5.3. SUMMARY

The average LD_{50} observed in different animal experiments indicates that the oral dose values range from 400-830 mg Mn/kg of soluble manganese compounds, much higher than the 38-64 mg Mn/kg for parenteral injection. The toxicity of manganese varies with the chemical form in which it is administered to animals. Acute poisoning by manganese in humans is very rare. It may occur following accidental or intentional ingestion of large amounts of manganese compounds. Along with a number of other metals, freshly formed manganese oxide fumes have been reported to cause metal fume fever.

6. TOXIC EFFECTS AFTER CHRONIC EXPOSURE

6.1. INTRODUCTION

Manganese exposure can produce prominent psychological and neurological disruptions. These manifestations of neurotoxicity are described below. The neurologic signs and symptoms have received particularly close attention because they resemble several other clinical disorders and, in particular, Parkinsonism and dystonia. Collectively, these disorders have been described as involving "extrapyramidal motor system dysfunction" because they result in damage within the extrapyramidal motor system and especially in the neostriatum, substantia nigra and, in the case of dystonia the thalamus (Figure 6-1). As a consequence of such damage, a constellation of signs and symptoms which disrupt the initiation, completion and smooth performance of motor acts arises. These frequently include tremor, jerkiness of movement, limb rigidity and postural disorders. While some controversy exists in the scientific literature concerning whether manganism is a better model of Parkinsonism or dystonia the principal value of such comparisons lies in the formation of hypotheses concerning the target of manganese neurotoxicity which can then be tested experimentally and which may ultimately assist in determining the no-effect level in animal species.

Comparison of manganism and Parkinsonism has been important in one other respect. Based upon similarities of symptoms, the principal therapy for Parkinsonism, administration of the drug, levadopa (l-DOPA) has also been applied to chronic manganese intoxication with some success.

Extensive laboratory research has been conducted to investigate the neural circuit which is damaged in Parkinsonism and which is a presumed target of manganese neurotoxicity. This circuit consists of nerves which connect the substantia nigra and the neostriatum (Figure 6-2). These nerves

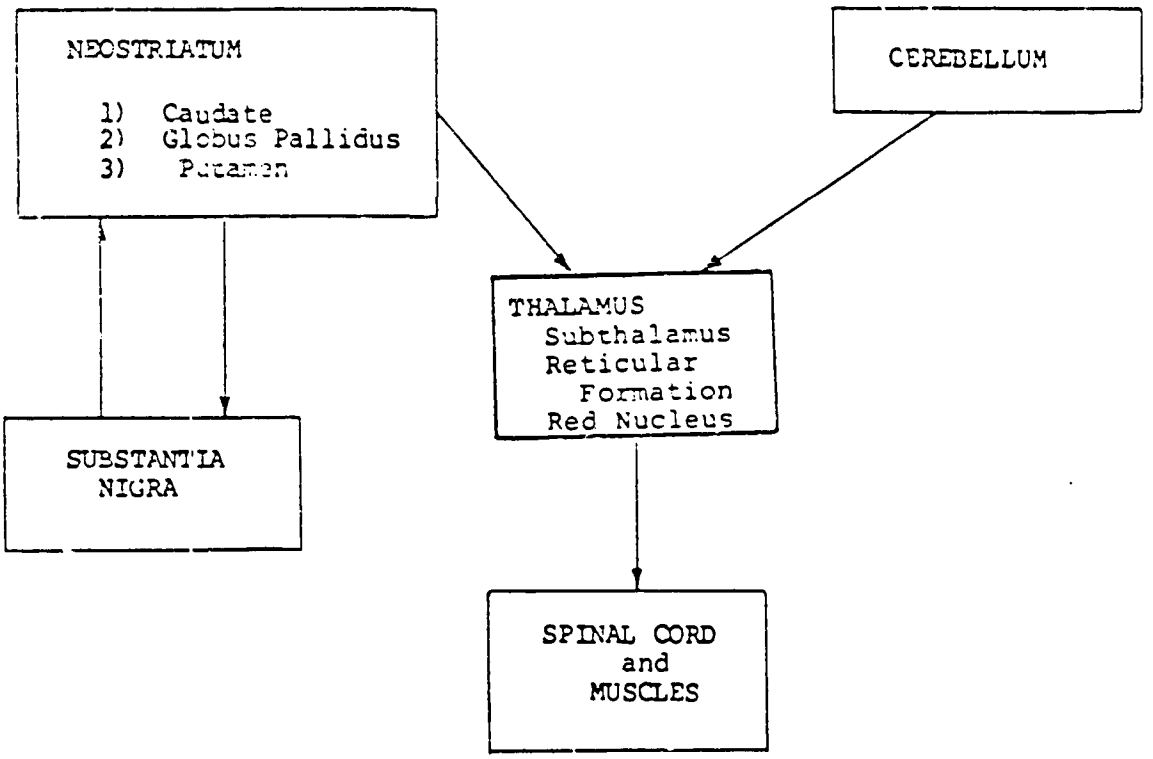

FIGURE 6-1

Principal Components and Connections in the Extrapyramidal Motor System

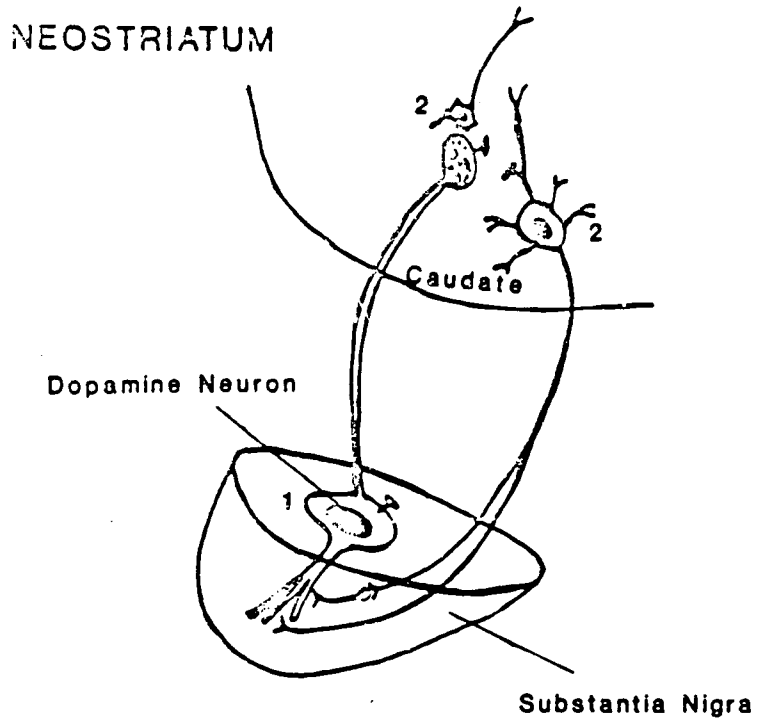

FIGURE 6-2

Schematic Illustration Depicting Possible Sites of Damage to the Nigral-Striatal System in Parkinsonism and Manganism

Source: Adapted from Cooper et al., 1982

contain the neurotransmitter, dopamine, and have been shown to sustain injury in Parkinsonism. In fact, l-DOPA is the immediate chemical precursor of dopamine and the simplest explanation of its effectiveness in Parkinsonism is based on the notion of replacement of dopamine available for neurotransmission. As indicated by both histopathologic and neurochemical studies conducted in animals it is unlikely that manganese produces the same neurological damage as Parkinsonism. Rather, attention has been focused upon nerve cells which are normally stimulated by the dopamine-containing neurons that project to the neostriatum and upon nerve cells which mediate the activity of the dopamine-containing neurons. This section also describes the different hypotheses which have been proposed to account for the manifestations of manganese neurotoxicity.

6.2. NEUROTOXIC EFFECTS - HUMAN STUDIES

The effect of manganese on the CNS is quite serious in the advanced form known as manganism. According to Voss (1939) there were 152 cases of manganism described in the literature prior to 1935. By 1943, Fairhall and Neal (1943) found 353 cases of manganese poisoning. Subsequently, reports of at least 200 additional cases of manganism have been published.

The signs and symptoms of chronic manganese poisoning have been described in detail several times (Flinn et al., 1940; Ansola et al., 1944a,b; Penalver, 1955; Rodier, 1955; Schuler et al., 1957; Chandra et al., 1974). This poisoning can result from exposure to manganese aerosols after only a few months, although it usually results from exposures of 2-3 years or longer (Ansola et al., 1944b; Rodier, 1955). It has been suggested that damage is reversible if the patient is removed from exposure at an early stage. On the other hand, once profound neurologic signs and symptoms are present they tend to persist and may even worsen several months after

exposure has ceased (Barbeau et al., 1976). This finding is corroborated by Cotzias et al. (1968) who reported that the presence of elevated tissue manganese concentrations was not necessary for the continued neurologic manifestations of manganese poisoning.

Human manganese intoxication produces signs and symptoms of central nervous system toxicity which can be divided into two broad stages, the first dominated by psychological disturbances which subside if manganese exposure is terminated and a second, predominantly neurological disturbance, which occurs with continued manganese exposure and which is not reversible.

The disease begins insidiously with anorexia, asthenia, and occasionally psychotic behavior [the latter-most reported most frequently in studies of manganese miners than those from other occupational categories (Table 6-1)]. Severe somnolence followed by insomnia is often found early in the disease. Headache and leucopenia may further confuse the differential diagnosis between manganism and viral encephalitis.

As manganese exposure continues, slurred speech, a mask-like face and general clumsiness with loss of skilled movement are characteristic. Indifference occurs, interrupted by spasmodic laughter or by crying spells (Table 6-2).

A more specific description of the earlier stages of this disorder has appeared in conjunction with a report of cases in the United States (Cook et al., 1974). Symptoms were consistent with the literature except for the absence of "manganese psychosis." The most characteristic signs were the various gait disorders. Six cases showed similarity in the earliest symptoms: somnolence, incoordination, speech disorder, gait difficulty, and imbalance. Postural tremor and tremors at rest were seen in four of the six cases. In no case was this tremor the only symptom and all four had slurred speech, asthenia and somnolence.

TABLE 6-1

Psychological Disturbances in 15 Cases of Manganism*

Type of Disturbance	Disturbances in Each Case															Cases in Which Each Disturbance Occurred	
																Number	Percent
Emotional instability	+	+	+	–	+	+	+	+	–	–	–	–	–	–	–	8	53
Irritability	+	+	+	–	+	+	+	+	–	–	–	–	–	–	–	7	
Restlessness	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	4	
Tendency to weep	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	3	
Withdrawal from group	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	3	
Unmotivated laughter	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	1	
Apathy	+	–	+	–	–	+	–	+	+	+	+	–	–	–	–	7	47
Hallucinations	+	–	+	–	–	–	–	–	+	+	–	+	+	–	–	6	40
Flight of ideas	+	–	+	+	–	+	–	–	–	–	–	–	–	+	–	5	33
Compulsory acts	+	–	–	+	+	–	–	–	–	–	+	+	–	–	–	5	33
Verbosity	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	4	27
Total number of disturbances each case	9	6	5	4	4	3	2	2	2	2	2	2	1	1	0		

*Source: Schuler et al., 1957

Neurological Symptoms in [illegible] Manganism*

Symptom	Symptoms in Each Case															Cases in Which Each Symptom Occurred	
																Number	Percent
Asthenia and adynamia	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	14	93
Sialorrhea	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	13	87
Fatigability	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	13	87
Cephalalgia	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	12	80
Disturbances of sleep	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	12	80
Muscle pains	+	+	-	+	+	+	+	-	+	-	+	+	-	-	+	10	67
Paresthesias	+	+	+	-	+	[illegible]	+	+	+	-	+	+	-	-	-	9	60
Diaphoresis	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	6	40
Disturbances of speech	+	-	+	+	-	+	-	+	-	-	-	-	-	-	-	5	33
Disturbances of libido	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	4	27
Disturbances of ejaculation	+	+	+	-	-	-	-	-	-	-	-		-	-	-	3	20
Total number of symptoms in each case	11	10	10	9	8	8	7	7	7	5	5	4	4	3	3		

*Source: Schuler et al., 1957

Fully developed manganism causes severe rigidity with the extremities showing the "cogwheel" phenomenon in which passive movement of the limbs results in resistance and jerky cog-like rather than smooth movement. Tremors may occur which become exaggerated by emotion, stress, fatigue or trauma. Similarly, an autonomic disturbance manifested by excessive salivation and sweating may become apparent (Table 6-3). These latter symptoms are persistent.

Symptoms and signs of chronic manganese poisoning have often been compared to Parkinson's disease, but certain differences should be noted. Parkinson's patients show pronounced disturbances of motor behavior which include tremor observed at rest rather than during an intentional motor act as in manganism (Klawans et al., 1970). Parkinson's patients also exhibit difficulty initiating and stopping motor acts, expressionless face and hypoactivity. While Parkinsonism may be associated with psychological disturbances such as depression and occasionally psychotic behavior, these are not considered common manifestations of the disorder. Barbeau et al. (1976) provide a revised description suggesting that chronic manganese poisoning is a better model of another extrapyramidal disorder, dystonia, than of Parkinson's disease. They point out that the tremor observed in some of the patients with manganese poisoning is quite different from that seen in Parkinson's disease. In their opinion it has much more of an attitudinal or flapping quality. These authors note that some form of dystonia, defined as a postural instability of complementary muscle groups, is an almost obligatory feature of manganism. However, dystonia is an extremely broad diagnostic category and both its manifestations and histopathology show large variability among patients.

TABLE 6-3

Neurological Signs in 15 Cases of Manganism*

Signs	Signs in Each Case																Cases in Which Each Sign Occurred	
																	Number	Percent
Muscular hypertonia	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	13	87
Expressionless face	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-		10	67
Gait changes	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-		10	67
Monotonous voice	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-		7	47
Tremor, upper extremities	+	+	+	-	-	+	-	-	+		-		-	+	-		7	47
Tremor, lower extremities	+	+	+	+	-	+	-	-	+	-	+	-		-	-		6	40
Superficial sensory disturbance	+	+	+	+	-	-	-	-		-		-	-	-	-		4	27
Deep sensory disturbance	+	+	+	-	+	-	-	-		-		-	-	-	-		4	27
Impaired hearing	-		-	+	-	-	-	-	-		-	+	-	+	-		3	20
Postural changes	+	+		-	-	-	-	-		-		-	-	-	-		2	13
Diplopia	-	+	-	-	-	+	-	-	-		-		-	-	-		2	13
Pyramidal signs	-		-	-	+	-	+	-	-		-		-	-	-		2	13
Total number signs in each case	9	9	8	7	6	6	5	4	4	3	2	2	2	2	1			

*Source: Schuler et al., 1957

The clearest basis for distinguishing Parkinsonism and manganism is on histopathological evidence. The classical findings in Parkinson's disease are depigmentation and loss of cells in the substantia nigra, locus coeruleus and dorsal nucleus of the vagus with little damage to the striatum or pallidum (Figure 6-3). In chronic manganese poisoning there is no appreciable destruction of the substantia nigra; the lesions are found mainly within the striatum and pallidum (see Figure 6-3).

However, on the basis of the similarity in clinical signs to Parkinsonism, treatment with levadopa has been attempted in established manganism with success in some cases (Barbeau et al., 1970; Rosenstock et al., 1971). This finding adds credence to the belief that an essential aspect of manganese neurotoxicity is depletion of function in dopamine-containing

6.2.1. Case Reports and Epidemiologic Studies. Reports of cases of manganism and the clinical descriptions have established that exposure to manganese can cause chronic manganese poisoning in some individuals. In order to establish levels of exposure at which effects do not occur it is necessary to have adequately clearly described levels of exposures (including specific chemical form, particle size). Additionally, the number and selection of individuals exposed and studied should be clearly defined. Although there has been a good deal of occupational exposure to manganese, this type of dose/response data is not available. There are, however, many reports of cases of manganism including a few in which an identified exposed group has been examined for early signs of the disease. The studies which have been reviewed with the goal of identifying the no-observed-effect level (NOEL) are discussed. However, the cross-sectional approach of most of the studies introduces selection biases, including the concern that disabled individuals may have been lost from the

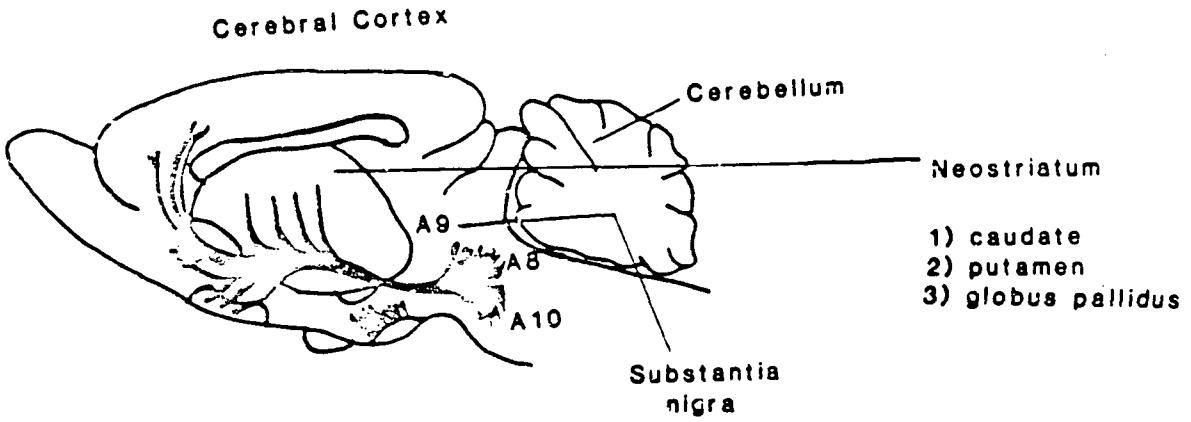

FIGURE 6-3

Schematic diagram indicating the distribution of the main central neuronal pathways containing dopamine. The stippled regions indicate the major nerve terminal areas. The cell groups in this figure are named according to the nomenclature of Dahlström and Fuxe (1965).

Source: Adapted from Cooper et al., 1982

work force and excluded from the studies. Despite these limitations, there are human studies which, taken together, define a range of lowest-observed-effect levels (LOEL).

Manganism has been described in workers in ore crushing and packing mills, in ferroalloy production, in the use of manganese alloys in the steel industry, in the manufacture of dry cell batteries, and in welding rod manufacture. Exposure typically involved dusts of manganese oxides generally larger than 5 μm, or fumes produced through vaporization and subsequent condensation with particle size of 0.1-1 μm, but information on manganese concentrations and the occurrence of other chemicals at working places was usually limited. Few studies dealt with the particle size distribution of manganese aerosols.

Most of the described cases of manganism occurred in manganese mines. The reported poisonings were among Huelva miners in Spain (Dantin Gallego, 1935, 1944), Sinai miners (Nazif, 1935; Scander and Sallam, 1936), miners from Giessen in Germany (Büttner and Lenz, 1937), Moroccan miners (Baader, 1939; Rodier and Rodier, 1949), Chilean miners (Ansola et al., 1944a,b), Cuban miners (Garcia Avila and Penalver, 1953), Suceova miners in Rumania (Wassermann et al., 1954), Mexican miners (Roldan, 1956), USSR miners (Khazan et al., 1956; Khavtasi, 1958), Japanese miners (Suzuki et al., 1960), and Indian miners (Balani et al., 1967).

Table 6-4 contains a summary of those studies with corresponding exposure data and a description or response frequency for CNS involvement in workers occupationally exposed to manganese by inhalation. These studies are presented in chronological order. The earlier ones in particular have several limitations due in part to the fact that they were designed to obtain clinical information rather than incidence or prevalence rates. The

TABLE 6-4

Studies of Manganism in Humans and Exposure-Response Relationship

Type of Exposure	Chemical (particle size)	Exposure Level (mg Mn/m³)	Duration of Exposure	Number Affected/ Number Studied	Signs and Symptoms[a]	Reference
Ore crushing mill/dust	oxides, mostly MnO_2 (NR);	10-30 30-180	3.3 year average	0/9 11/25	none 44% manganism	Flinn et al., 1941[c]
Manganese mine	NR	62.5-250	178 days	12/12	manganism	Ansola et al., 1944a,b
Manganese mine; dusts	NR (90% <5 μ)	250-450	~1 month to 10 years	NR	150 cases manganism	Rodier, 1955
Manganese mine	oxides (NR)	1.5-16[b]	8.2 year average (9 months to 16 years)	15/83	manganism	Schuler et al., 1957
Industrial plants	NR	<5 5-30	NR	0/38 7/117	none 6% manganism	Tanaka and Lieben, 1969
Dry-cell battery industry; dusts	65% MnO_2 (NR)	6.8-42.2[b]	7.5 year average (1-16 years, cases)	8/36	22.2% manganism psychosis	Emara et al., 1971
Ferromanganese production and processing	ferromanganese, small amounts of MnO, Mn_3O_4 (95% <5 μ) and/or 100% Mn oxides, mainly Mn_3O_4 (<2 μ)	2.1-12.9 and/or 0.12-13.3	8-26 years in five cases	5/71	7% manganism	Smyth et al., 1973
Ferromanganese industry	NR (0.5-6 μ; mostly 4.5)	0.06-4.9	12 years (12 hours/day)	26/160	30% subjective symptoms; 2% "health disorders due to manganism"; symptoms increased with number of years of employment	Suzuki et al., 1973a
Ferromanganese industry: electric furnace workers	NR (<1.5 μ)	3.2-8.6	8.5-6.8 years	40/100	40% subjective symptoms; 8-10% single neurological signs, e.g., tremor of fingers	Suzuki et al., 1973b
Ferromanganese plant, dust and fumes	NR	0.30-20.44	27% <4 years 9.8% >20 years	62/369	16.8% slight neurological signs, e.g., tremor at rest, pathological reflexes	Saric et al., 1977[c]

TABLE 6 4 (cont.)

Type of Exposure	Chemical (particle size)	Exposure Level (mg Mn/m³)	Duration of Exposure	Number Affected/ Number Studied	Signs and Symptoms[a]	Reference
Control I electrode plant		0.002-0.030 (emissions from ferromanganese plant)	NR	11/190	5.8% neurological findings	Saric et al., 1977
Control II aluminum rolling mill (ambient levels)		≤0.07 µg/m³	NR	0/204	none	Saric et al., 1977
Welding fumes	NR	0.44-0.99[d]	20.2 (mean year) (10-31)	5/20	25% slight neurological signs (brisk deep reflexes)	Chandra et al., 1981
		0.5-0.8[d]	21.9 (mean year) (2-32)	10/20	50%	
		0.88-2.6[d]	14.1 (mean year) (6-27)	9/20	45%	

[a]Percentage is given if sample has been selected such that the rate can be considered an estimate of prevalence

[b]Range of averages for different areas or workstations sampled

[c]See also Tables 6-5 and 6-6

[d]In workers breathing zone

NR = Not reported

developing exposure standards. Generally the exposure data covers a broad range and does not include particle size or chemical characterization. In some cases exposures change over time (e.g., Flinn et al., 1941; Smyth et al., 1973). The selection and composition of the exposed group may not be adequately described or may be based on high exposure. None of these studies employs a standard cohort design. Duration of exposure is sometimes presented only for diagnosed cases, and the endpoints differ among studies. Many clinical examinations are poorly standardized and results are rarely subjected to statistical analysis. Percentages reported in the table reflect prevalence of the pathological findings in the group as described. While the use of this information for obtaining a dose-response association is limited quantitatively, it does show evidence of effects in humans and can be used to broadly estimate a range of LOELs.

Flinn et al. (1941) examined 34 manganese exposed workers representing all of the exposed individuals from the same ore crushing mill. The authors described the 23 workers without chronic manganese poisoning as exposed but not affected. However, Table 6-5 shows that these workers had some neurological findings which might be indicative of early manganism. The average exposure for those affected was 5.3 years and for the exposed workers unaffected was 2.4 years. No case of manganism was detected in nine workers exposed to average manganese concentrations of 10-30 mg/m^3 in two manganese ore crushing mills (Flinn et al., 1940). The lowest average manganese concentration at which the disease was found was 30 mg/m^3. However, only two of these nine men were exposed for more than 3 years. Althougn the entire exposed group was examined, the numbers are small and exposures too short to define 16-30 mg/m^3 as a NOEL.

TABLE 6-5

Frequency of Abnormal Neurological Findings[a]

	Percentage			Number of Cases		
	Affected	Nonaffected	Nonexposed	Affected	Nonaffected	Nonexposed
Total examined				11	23	16
Tremor of tongue	91	35	19	10	8	3
Gait disturbances	90[b]	0	0	9[b]	0	0
Speech disturbances	73	0	0	8	0	0
Tremor of extremities	55	22	0	6	5	0
Muscular weakness	55	0	0	6	0	0
Intention tremor of hands	45	17	0	5	4	0
Abnormal reflexes	45	35	31	5	8	5
Masked facies	45	0	0	5	0	0
Sensitive achilles tendon	36	4	0	4	1	0
Abnormal handwriting	27	0	0	3	0	0
Spasm of extremities	18	0	0	2	0	0
Lateral nystagmus, slight	18	17	0	2	4	0
Abnormal psyche	18	4	0	2	1	0

[a]Source: Flinn et al., 1941

[b]Excluding Case No. 64 who was too weak to walk normally due to a previous illness

In 1933, Roller reported 150 cases of manganism from three Moroccan mines. Underground workers engaged in drilling blast holes ran a high risk of developing manganese poisoning; 132 of 150 cases occurred among workers using the drills and the other cases were laborers who worked nearby. Concentrations of manganese were usually very high in the mines from which cases of manganism were reported. The manganese concentration in the air in the immediate vicinity of rock drilling in Moroccan mines was ~450 mg/m³ in one mine and ~250 mg/m³ in another. Analyses of the ores indicated that toxicity was not strictly related to manganese content; most of the cases of manganism resulted from exposure to an ore from one mine that was less oxidized than the ores from the other mines. In two reports from Chilean mines (Ansola et al., 1944a,b; Schuler et al., 1957) the concentrations of manganese in the air varied from 62.5-250 mg/m³ and from an average of 1.5-16 mg/m³, respectively. Schuler et al. (1957) observed that the introduction of pneumatic drilling and the associated increase in dust led to outbreaks of manganism. The investigators did not examine all of the workers and stated that their study was not designed to provide incidence data. The total number examined was ~83 and the procedure for selecting them was not described. Therefore, prevalence rate was not applicable.

Emara et al. (1971) studied 36 workers exposed to manganese dioxide dust in a factory manufacturing dry batteries. Average concentrations ranged from 6.8-42.2 mg Mn/m³ in four areas. Eight workers (22%) exhibited symptoms of manganism. Concentrations at the main working areas of three of the cases ranged from 6.2-7.2 mg/m³. Cases had been working 1-16 years prior to diagnosis of chronic manganese poisoning.

After an industrial hygiene survey identified certain plants in Pennsylvania as having manganese exposures above the threshold limit value (TLV) of

5 mg/m³, Tanaka and Lieben (1969) selected factories with and without such exposures, and examined workers in the selected factories. All four plants processing manganese ore or ferromanganese had samples above the TLV as did 60% of chemical manufacturing plants. Neurological screening of 117 workers from the factories where exposures >5 mg/m³ had been detected (81% of those exposed) revealed seven cases with "definite signs and symptoms of manganese poisoning." This study does not support a lack of effect at exposures <5 mg/m³ due to lack of standardized examination procedures, explanation of selection patterns, details on industrial exposures, duration of exposure, and the small, unrepresentative sample in the low exposure group. The only exposure levels presented were for the two case histories described.

Smyth et al. (1973) performed repeated sampling and analysis of the manganese concentration around 15 work positions in a ferromanganese alloy processing plant. They selected 71 employees for study who were exposed daily in areas involving these work positions. Another group of 71 unexposed male employees matched by age and length of plant service were selected as controls. The weighted average concentrations for manganese in air ranged from 0.12-13.3 mg/m³ for fumes and from 2.1-12.9 mg/m³ for manganese dust. However, all cases were probably exposed to the high average dust concentrations which had been recorded in previous years (30 mg/m³). The authors reported a poor correlation between manganese exposure and manganese excretion in the urine. This may not be surprising as manganese elimination occurs primarily via biliary excretion. Fecal manganese content may have provided a better correlate to manganese exposure. Five exposed individuals and no controls had signs suggestive of early manganism. Three of these cases had several classical signs such as masked facies, but the other two

had only loss of associated arm movements bilaterally. The detailed exposures by position were not explained on a case by case basis and therefore could not be associated with each individual. Exposure duration in the five cases ranged from 8-26 years although it is not known when signs of manganism first appeared.

Saric et al. (1977) compared 369 workers exposed to 0.3-20 mg Mn/m³ at a ferroalloy plant to two other groups; 190 workers at an electrode plant exposed to 0.002-0.03 mg/m³ (2-30 µg/m³) and 204 workers at an aluminum rolling mill exposed to ambient levels <0.0001 mg/m³ (<0.10 µg/m³). Neurological examinations were given to 95% of all workers. Prevalence of neurological signs was 17% in workers in the ferroalloy plant, compared to 6% and 0% of workers in the electrode and aluminum plant, respectively. The most prevalent symptom, tremor at rest, is not unique to manganese, therefore all cases cannot be definitely attributed to exposure to manganese. There was no apparent association of neurological symptoms with smoking habit. The ferroalloy workers were further categorized into three groups by mean manganese concentrations at working places: <5 mg/m³, 9-11 mg/m³, and 16-20 mg/m³. In addition to manganese compounds, carbon monoxide, carbon dioxide and coal dust were also present. Table 6-6 summarizes neurological signs observed in these groups. These data suggest that slight neurological disturbances may occur at exposures <5 mg/m³ and seem to be more prevalent at higher exposures.

Chandra et al. (1981) reported on three groups of 20 welders each exposed to levels <3 mg/m³ compared to 20 controls. The welders were exposed to manganese released from manganese-coated electrodes as well as from materials being welded. The materials being welded were stated to be mainly steel; no data were given on exposure to other metals. The groups

TABLE 6-6

Ferroalloy Workers with Neurological Signs by Level of Exposure to Manganese[a]

Signs	Mean Manganese Concentrations at Working Places (mg/m³)			
	0.301-4.933 (N = 268)	9.480-11.062 (N = 17)	16.347-20.442 (N = 18)	Total (N = 303)[b]
Cogwheel phenomenon	1	0	0	
Difficulty in initiating voluntary movements	2	0	0	
Pathological reflexes	6	1	1	
Tremor at rest	42	2	2	
Pathological reflexes and tremor at rest	3	0	0	
Cogwheel phenomenon and tremor at rest	0	0	1	
Cogwheel phenomenon and pathological reflexes	0	0	1	
Total	54 (20.1%)	3 (17.6%)	5 (27.8%)	62 (16.8%)

[a]Adapted from Saric et al., 1977

[b]Total number examined was 369. The authors state in a footnote that "in 66 workers with a mean manganese exposure of 0.469-1.056 mg/m³ no neurological signs were found". It seems that these should be included in the low exposure group in this table, in which case the prevalence of signs for this group is 16%.

came from a heavy engineering shop, a railway workshop, and a ship repair shop. Many workers had been employed for >10 years. Means of airborne manganese were stated to average 0.31, 0.57 and 1.75 mg/m³ with slightly higher ranges in the workers' breathing zone (see Table 6-4). No data were given on particle size, but it can be assumed that both fumes and small particles were inhaled. Positive neurological signs were reported to occur in the form of brisk deep reflexes of the arms and legs and tremors of the hand and tongue in 25, 50 and 45% in these three groups respectively, whereas none of the controls showed such effects. No details of the neurological examination were presented. The mean exposure times of these groups were 20, 21 and 14 years, respectively. No statistical analysis or analysis by person-years of exposure was presented.

Sabnis et al. (1966) assessed average daily exposure to manganese in a ferromanganese alloy factory in India. The daily average weighted exposures were <2.3 mg/m³ for all workers in the factory, although maximum levels were recorded up to 10 mg/m³. The medical officer of the factory reported that he had observed neither acute nor chronic cases of manganese poisoning among the workers. A list of subjective symptoms of manganism was prepared for the medical officer who stated that no worker had reported such symptoms, but this list was not included in their report. This data cannot be used to identify a NOEL because no clinical examinations were performed. Other reports suggest that signs of manganism can be identified in individuals not experiencing symptoms (e.g., Smyth et al., 1973).

Sabnis et al. (1966) relate in their report that manganese poisoning had occurred in a nearby factory. High levels of 8.8 and 8.4 mg/m³ occurred at operations here compared to 2.7 and 2.3 mg/m³ recorded in the ferromanganese alloy factory which had no reports of poisoning. Duration of

exposure was not reported at either factory. The authors concluded that 6 mg/m^3 (the standard in effect at that time) was unsafe and that daily weighted exposures up to 2.3 mg/m^3 were safe.

While the above studies do not show a clear dose-response relationship, they do support the association of neurological symptoms and signs with exposure to manganese.

6.2.2. Pathology of Manganese Poisoning. Pathologic findings observed at autopsy have ranged from absence of morphologic changes, through specific lesions of the neostriatum, to generalized pathology of both the central and peripheral nervous systems (Casamajor, 1913; Ashizawa, 1927; Canavan et al., 1934; Stadler, 1936; Trendtel, 1936; Voss, 1939, 1941; Flinn et al., 1941; Ardid and Torrente, 1949; Parnitzke and Pfeiffer, 1954; Bernheimer et al., 1973; Barbeau et al., 1976). The most extensive degenerative changes have been found in the neostriatum (caudate nucleus, putamen and pallidum) and evidence indicates that the pallidum may be preferentially damaged.

6.2.3. Summary. An important effect of chronic exposure to manganese is the chronic manganese poisoning resulting from occupational exposures to manganese dusts after only a few months of exposure, although other cases develop only after many years. Earlier studies report advanced cases of manganism (in various miners), but more recent studies report cases showing neurological symptoms and a few signs where the exposure was at much lower concentrations. Whether this reflects different chemical form and particle size of the inhaled manganese, a straight dose-response effect or inconsistencies in clinical examination is not clear.

The human studies are not adequate to identify a dose-response relationship, but do permit the identification of the LOEL. The full clinical picture of chronic manganese poisoning is reported less frequently at

exposure levels below 5 mg/m³ (Saric et al., 1977; Chandra et al., 1981; Tanaka and Lieben, 1969; Sabnis et al., 1966). The studies reporting effects at the levels reported by Chandra et al. (1981) and of Saric et al. (1977) describe effects which cannot be definitely attributed to manganese. Saric et al. (1977) report tremor at rest as the major effect on workers in the electrode plant exposed to 2-30 µg/m³ (0.002-0.03 mg/m³) although duration of exposure was not fully detailed. The prevalence of a few signs in workers exposed to 0.3-5 mg/m³ (Saric et al., 1977) and 0.4-2.6 mg/m³ (Chandra et al., 1981) suggest that the LOEL may range to as low as 0.3 mg/m³ (300 µg/m³). The data available for identifying effect levels below this level is equivocal or inadequate. This is further complicated by the fact that good biological indicators of manganese exposure are not presently available. Consequently, studies directed toward clearly defining the dose-effect relationship will undoubtedly facilitate a more realistic estimate of the risk to developing manganism. There is no clear-cut evidence of chronic manganese poisoning under 5 mg/m³.

The broad exposure ranges, the incomplete descriptions of chemical form and particle size are insufficient to relate response to exposure characteristics. The exposure data reported by Smyth et al. (1973) suggests that ferromanganese fumes may have a smaller particle size than the dusts and thus more respirable particles.

In order to obtain definitive dose response data, a cohort study is needed, including documented clinical examinations, more accurate exposure characterization as well as exposure data on individuals. All members of the cohort should be followed for neurological signs for at least 20 years and numbers lost to follow up should be clearly reported.

6.3. NEUROTOXIC EFFECTS - ANIMAL STUDIES

The wide range of epidemiological studies indicates that the clinical manifestations, observed morphological lesions and biochemical changes described in chronic manganese intoxication closely resemble those that occur in other extrapyramidal disorders, notably Parkinsonism. The exact mechanism of biochemical changes is still debated, as is the role of manganese in the extrapyramidal syndrome in exposed workers (Barbeau et al., 1976; WHO, 1981). Such controversy regarding the neurological component of chronic manganese intoxication in exposed workers prompted a wide range of animal studies focused on the neurotoxic effects of this metal.

Most of the earlier neurologic studies in animals utilized the parenteral or respiratory route of administration. Table 6-7 summarizes some of the more recent data on neurological effects. An in-depth analysis of all available animal data suggests that no accurate dose-response relationship for neurological effects of chronic manganese exposure can be assessed since the methodology and reported values vary significantly among investigators. For instance, very few of the early studies reported brain levels of manganese (Pentschew et al., 1963; Neff et al., 1969; Mustafa and Chandra, 1971; Bonilla and Diez-Ewald, 1974; Sitaramayya et al., 1974). In some of the more recent studies where the brain manganese levels are reported, the results obtained by different workers do not always agree. In some studies (Chandra et al., 1979a; Chandra and Shukla, 1981) the brain manganese concentrations were reportedly an order of magnitude higher than those obtained by most workers (Underwood, 1977; Bonilla, 1978, 1980; Deskin et al., 1981a; Chan et al., 1981; Lai et al., 1981b, 1983c). Furthermore, in recent studies by the same group (Chandra et al., 1979b; Murthy et al., 1981) the brain manganese levels are reportedly different from values in their other studies (Chandra et al., 1979a; Chandra and Shukla, 1981).

TABLE 6-7

Neurotoxic Effects of Manganese in Experimental Animals

Species	Compound	Route	Dose (mg Mn/kg)		Duration in Months	CNS Abnormality[a]			Reference
			Single	Total		Behavioral	Histological	Biochemical	
Rat	$MnCl_2 4H_2O$	i.p.	2.2	535	8	+	NS	NS	Roussel and Renaud, 1977
Rat	$MnCl_2 4H_2O$	i.p.	2.2	401	6	-	+	NS	Chandra and Srivastava, 1970
Rat	$MnCl_2 4H_2O$	i.p.	2.2	268	4	NS	NS	+	Sitaramayya et al., 1974
Rat	$MnCl_2 4H_2O$	i.p.	4.2	189	1.5	NS	NS	+	Shukla and Chandra, 1977
Rat	$MnCl_2 4H_2O$	i.p.	4.0	120	1	-	+	+	Chandra et al., 1979b
Rat	$MnCl_2 4H_2O$	i.p.	4.2	63	1	-	+	+	Shukla and Chandra, 1976
Rabbit	MnO_2	i.t.	169.0	169	24	+	+	+	Chandra, 1972
Rhesus monkey	MnO_2	i.m.	125, 220	345[b]	9 14	+ +	NS +	NS NS	Pentschew et al., 1963
Squirrel monkey	MnO_2	s.c.	250.0	500[c]	3	+	-	+	Neff et al., 1969
Monkey	MnO_2	s.c.	39.5 79.0 158.0	355[d] 711[d] 1422[d]	2 1.5 1	+ + +	- - -	NS NS NS	Suzuki et al., 1975
Rats and monkeys	Mn_3O_4	Inhalation		11.6[e] 112.5[e] 1152.0[e]	9	-	-	NS	Ulrich et al., 1979a,b,c

[a]NS = Not studied

[b]Doses 2 months apart. Each dose was spread over eight injection sites.

[c]Doses 1 month apart

[d]Nine weekly doses

[e]Continuous 24 hours/day exposure. Units are in μg Mn/m³.

There is also concern about the appropriateness of certain animal species in studying manganese toxicity. The available evidence obtained with small laboratory animals indicates that rats may display some of the neurobiochemical changes associated with manganism in humans but they do not exhibit the wide range of behavioral manifestations described in primates (Chandra and Srivastava, 1970; Chandra et al., 1979a,b; Singh et al., 1974, 1975; Shukla and Chandra, 1976, 1977; Sitaramayya et al., 1974). This lack of effect seen in the rat may not be specific to manganese toxicity. In attempting to develop animal models of Parkinsonism and other extrapyramidal dysfunctions, small laboratory animals have not been found to show similar behavioral pathologies (e.g., tremor, akinesia, gait disorders). As a consequence of this, studies conducted in rodents have tended to rely on what might be homologous behaviors. The accuracy with which such studies model the disorder observed in primates is open to some question.

There may be additional reasons to favor primate over small laboratory animal studies of manganese toxicity. Manganese accumulation appears to be relatively high in pigmented tissues. Since the primate, but not rodent substantia nigra shows pigmentation, there is some basis for predicting species differences in accumulation and, consequently toxicity, of manganese.

Histopathologic studies of manganese toxicity in small animals have found scattered neuronal degeneration in the cerebral and cerebellar cortex (Chandra and Srivastava, 1970; Chandra et al., 1979b; Shukla and Chandra, 1976), but have only occasionally observed changes in the neostriatum (Chandra, 1972). Consequently, with the exception of intratracheally exposed rabbits described below (Mustafa and Chandra, 1971, 1972; Chandra, 1972), studies with small animals did not find the characteristic histopathologic features of the extrapyramidal disease of manganism which are

prominent in exposed workers and which are presumed to be responsible for the behavioral manifestations of manganese intoxication.

It is probable that the signs of extrapyramidal disease are so subtle in some species that they cannot be noticed without special procedures. Therefore, Roussel and Renaud (1977) performed a study to determine if the human sleep disturbances observed in Parkinson's disease and in chronic manganese poisoning appear in the rat after chronic manganese intoxication. They found alteration of the sleep-wake cycle in rats exposed i.p. to 2.2 mg Mn/kg bw daily for 8 months. Chronic manganese intoxication in this experiment created an increase in slow-wave sleep and a decrease in paradoxical sleep by modification of the length of the phases. However, these changes can be attributed to disturbances in cortical activity rather than to lesions of the extrapyramidal system.

Experiments with rats indicate that a daily i.p. administration of 2-4 mg Mn/kg bw produces neuronal degeneration in the cerebral and cerebellar cortex and that a period of up to 120 days appears to be a threshold for the appearance of microscopic lesions (Chandra and Srivastava, 1970; Shukla and Chandra, 1976, 1977). These experiments also demonstrate that the maximum number of degenerated neurons is present when the amount of manganese in the brain is at maximum, thus indicating that the extent of damage to brain cells is directly related to the amount of manganese present (Chandra and Srivastava, 1970; Shukla and Chandra, 1977). Iron deficiency in the presence of treatment with manganese results in the highest levels of manganese in rat brain tissue. Some other studies have shown that biochemical changes (e.g., decreased activity of succinic acid dehydrogenase, increased activity of monoamine oxidase) may appear earlier than histological alteration of the brain, i.e., even 30 days after the beginning of manganese exposure

(Sitaramayya et al., 1974; Shukla and Chandra, 1976, 1977; Chandra et al., 1979a,b). However, from all these experiments performed on rats, it appears that the threshold for the appearance of microscopic lesions and biochemical changes occurs when the manganese in the brain reaches a level of ~4-5 µg/g of dry tissue (Singh et al., 1979).

Mustafa and Chandra (1971, 1972), and Chandra (1972) carried out an extensive study on rabbits intratracheally inoculated with 400 mg of MnO_2, corresponding to ~170 mg Mn/kg bw. After a period of 18-24 months, the inoculated rabbits developed paralysis of the hind limbs. The animals also showed a widespread neuronal loss and neuronal degeneration in the cerebral cortex, caudate nucleus, putamen, substantia nigra and cerebellar cortex. There was a marked decrease in brain catecholamines, particularly norepinephrine and dopamine, and a reduction in the activity of some enzymes in the manganese-dosed animals as compared with controls.

Primates are a better experimental animal than rodents for studying the neurological manifestation of manganese intoxication. Several studies with manganese dioxide-exposed monkeys have been performed (Mella, 1924; Neff et al., 1969; Pentschew et al., 1963; Suzuki et al., 1975), but all were conducted under inadequate experimental conditions (small numbers of animals were exposed to large, widely spaced doses of manganese by non-natural routes) (see Table 6-7). However, these exposures did consistently produce extrapyramidal symptoms (excitability, intention tremors, rigidity in the extremities) and/or histological lesions (damage to the putamen, caudate, subthalamic nucleus, and pallidum) that were remarkably similar to those described in cases of human manganism. Suzuki et al. (1975) administered s.c. injections of 0, 0.25, 0.5 and 1.0 g MnO_2 once a week for 9 weeks and found that the time of appearance of neurological symptoms and manganese

tissue concentrations in monkeys were proportional to cumulative dose (Table 6-8). Although the severity of symptoms was not dose-related, symptoms appeared earlier when higher doses were administered.

In contrast to the experiments described above, Ulrich et al. (1979a,b,c) observed no neurological or other pathological changes in groups of 8 squirrel monkeys and 30 Sprague-Dawley rats exposed to Mn_3O_4 aerosol at 11.5, 112.5 or 1152 µg Mn/m³ 24 hours/day (equivalent aerodynamic diameters ~0.11 µ). These three exposure groups and a control were exposed for 9 months and those not sacrificed observed for 6 additional months. No exposure-related effects on limb tremor or electromyographic activity were observed, although the techniques used to measure these parameters were described as sensitive enough to demonstrate differences if present. The authors report that there were no clinical signs of toxicity, but no details of the examination were presented. Histological examination of brain tissue for CNS alterations was reported to reveal no degenerative changes. These results indicate that large amounts of manganese may be required to produce extrapyramidal effects, since manganese levels in the blood of the monkeys exposed to the highest concentration were five times higher than in the controls after 9 months of exposure. Brain manganese levels were not reported.

Coulston and Griffin (1977) studied eight rhesus monkeys exposed continuously to 100 µg/m³ of Mn_3O_4 and observed daily for signs of toxicity. Six monkeys served as unexposed controls. After 12 months the authors report "no behavior or other visual manifestations of toxicity attributable to exposure to manganese" with no further details of the clinical examination. Two other rhesus monkeys exposed to 5000 µg/m³ of Mn_3O_4 for 23 weeks showed no signs of toxicity during the exposure period nor during a

TABLE 6-8

Neurological Signs Induced by Manganese in Monkeys[a]

Single Dose mg Mn (mg/kg)[b]	Time in Weeks and Cumulative Dose (mg Mn)							
	0	2	4	6	8	10	12	14
158 (39.5)	0	316	632	948	1264	1422		
					Tremor, excitability, choreiform movement, contracture of hand			
316 (79)	0	632	1264	1896	2528	2844		
				Tremor, excitability, choreiform movement, contracture of hand				
632 (158)	0	1264	2528	3792	5056	5688		
			Tremor, excitability, choreiform movement contracture of hand					

[a]Source: Adapted from Suzuki et al., 1975

[b]Dose per body weight not reported. Monkeys weighed 3.5-4.5 kg. Estimates are based on 4.0 kg animal.

10-month observation period. Examination of tissues showed no changes attributable to manganese.

The chronic toxicity of orally-administered manganese has not been adequately studied, but the available reports strongly suggest that it is very difficult, if not impossible, to produce the characteristic signs of extrapyramidal neurological disease in small laboratory animals exposed via drinking water or food. As discussed above, this may reflect fundamental species differences in response to disruption of neostriatal function. However, there is reason to expect that small laboratory animals may show neurochemical or other behavioral evidence of toxicity. Rats seem to be unaffected by dietary intakes as high as 2000 ppm (Wassermann and Wassermann, 1977). Kimura et al. (1978) reported that feeding with 2000 ppm of manganese chloride (564 ppm Mn) resulted in a slight decrease of the brain serotonin. Bonilla and Diez-Ewald (1974) exposed rats to 5000 ppm of manganese chloride (2180 ppm Mn) in drinking water, corresponding to ~306 mg Mn/kg bw. Despite the high manganese intake, none of the animals developed signs of extrapyramidal neurologic disease, such as muscular rigidity, tremor or paralysis of the limbs. Histopathological observation of the caudate nucleus revealed only moderate pyknosis of some neurons, and treated animals showed significant decreases in brain concentrations of dopamine and homovanillic acid. Bonilla (1978a,b) found an increase in the concentration of γ-aminobutyric acid in the brains of rats that were exposed to 10,000 ppm $MnCl_2$ in the drinking water (~600 mg Mn/kg bw) for 2 months.

Several recent experiments have been conducted to evaluate the effects of prolonged oral exposure to husmanite, manganous manganic oxide (Mn_3O_4), the major residue produced by heating MMT. The effect of chronic manganese oxide ingestion in rats maintained on a normal iron diet

(240 ppm Fe) and on a low iron diet (20 ppm Fe) was studied by Carter et al. (1980), Rehnberg et al. (1980, 1981, 1982) and Laskey et al. (1982). Animals were exposed to four different levels of Mn_3O_4 in their diet, 50, 400, 1100 and 3550 ppm manganese, corresponding to 2.25, 18, 50 and 160 mg Mn/kg bw, respectively. Animals treated with manganese and maintained on a normal iron diet or on a low iron diet did not develop signs of extrapyramidal neurologic disease, such as muscular rigidity, tremor or paralysis of the limbs. Recently, however, they have indicated (Gray and Laskey, 1980) that chronic dietary exposure to 1050 ppm manganese as Mn_3O_4, corresponding to ~140 mg/kg bw over a period of 2 months, reduces reactive locomotor activity (RLA) in mice and retarded growth of the testes and sex accessory glands. Whether the effects on activity and reproductive system development are causally related is uncertain.

Biochemical changes in the brains of rats exposed to 4.4 mg Mn/kg bw in their drinking water have been described (Singh et al., 1979), and similar exposure to 0.28 mg Mn/kg bw reportedly produced neuronal degeneration in the cerebral and cerebellar cortex of growing rats (Chandra and Shukla, 1978). Although dietary levels of manganese in the above studies were not reported, it is unlikely that the described changes are attributable to manganese exposure. It is important to note that the above doses are generally below the dietary level of ~20-30 mg Mn/kg bw that has been found to be optimal for development and growth in rats (Holtkamp and Hill, 1950; Hill and Holtkamp, 1954), and below the daily requirement for rats of 50 mg Mn/kg of diet (3-6 mg Mn/kg/day bw) that was recently recommended by the NAS (1978). Other recent studies relating biochemical changes in the brain to administration of manganese are discussed in the following section.

6.3.1. Mechanism of Manganese Neurotoxicity. After some five decades of research in this field, the mechanisms underlying the neurotoxicity of manganese and the pathogenesis of manganese encephalopathy have not been definitively elucidated. Several major factors contribute toward this lack of basic information: 1) the biological roles of this metal are not fully understood; similarly, there is an absence of a clear understanding of the pharmacokinetics, the homeostatic mechanisms as well as the deficiency - sufficiency - toxicity continuum of manganese; 2) the dose-effect relationship in manganese encephalopathy has not been systematically or adequately investigated; important variables such as the age, the species, the various forms of manganese and the routes of administration of manganese must be more seriously considered; 3) the neuroepidemiological data of human manganese encephalopathy are inadequate: the provision of complete data will undoubtedly generate new ideas and theories concerning the neurotoxic mechanisms underlying this syndrome (Silbergeld, 1982). However, despite the shortcomings just discussed, more recent studies employing animal models of this disease have provided some interesting and useful information such that a state-of-the-art evaluation of the possible and plausible mechanisms underlying the neurotoxic effects of manganese can be attempted. Since the dietary requirements of this metal for man and animals are relatively high (>40 ppm) (Underwood, 1977), the following discussion focuses primarily (although not exclusively) on studies where the administered manganese levels exceed the dietary requirement by at least two orders of magnitude.

A number of hypothetical mechanisms have been proposed to account for the neurotoxic effects of manganese causing the pathological and neurological changes in the CNS during manganese encephalopathy. However, when these mechanisms are considered within the framework of neurochemical

concepts, they can be grouped into two broad categories: 1) those that directly implicate altered neurotransmitter metabolism, and 2) those that do not directly involve dysfunctions of neurotransmitter systems, but also do not preclude the latter as being secondary, indirect or side effects.

6.3.2. Altered Neurotransmitter Metabolism.

6.3.2.1. EARLY PHASE OF RESEARCH IMPLICATING DISTURBANCES OF THE CENTRAL MONOAMINERGIC SYSTEMS -- Early neuropathological and histological findings reveal certain neuronal degenerative changes in the neostriatum, the subthalamic nuclei and less frequently in other brain regions in chronic manganese encephalopathy (Pentschew et al., 1963). From the more recent mapping studies (Ungerstedt, 1971) of the central monoaminergic systems in the mammalian CNS, it is apparent that some, if not most, of the neuronal degenerative alterations in manganese encephalopathy occur in the anatomical locations of these monoaminergic pathways (see Figure 6-3). Studies in human manganism as well as in animal models of this disease indicate that the levels of monoamines such as dopamine, noradrenaline and serotonin (and some of their metabolites) in the neostriatum are decreased (Neff et al., 1969; Mustafa and Chandra, 1971; Cotzias et al., 1971). Since these changes in the levels of monoamines also occur in Parkinsonism and since the clinical signs and symptoms of chronic manganese encephalopathy show many similarities with Parkinsonism, the hypothesis that the dysfunction of the central monoaminergic systems (particularly the dopaminergic system) was the underlying pathophysiological mechanism of chronic manganese encephalopathy was first proposed (Cotzias et al., 1971). Consistent with this hypothesis was the observation that treatment of patients with L-dopa, a classical anti-Parkinsonian drug, alleviates the symptoms of this disease (Mena et al., 1970).

6.3.2.2. RECENT STUDIES THAT SUPPORT THE HYPOTHESIS THAT THE CENTRAL DOPAMINERGIC SYSTEM IS DISTURBED IN CHRONIC MANGANESE TOXICITY -- Since the central dopaminergic system plays a key role in the normal functions of the basal ganglia, and dysfunctions of the basal ganglia are clearly discernible in chronic manganese encephalopathy, the proposal that a major, if not the major, neurotoxic effect of manganese involves disturbances of the central dopaminergic system appears most reasonable. Furthermore, the observations that in the human brain the manganese concentrations in the basal ganglia are higher than those in other regions (Curzon, 1975) and that in manganese-poisoned animals this brain region accumulates more manganese than other regions (Lai et al., 1981b, 1983a,c; Scheuhammer and Cherian, 1981; Chan et al., 1983) are consistent with this hypothesis. However, contrary data which do not provide evidence of greatest manganese accumulation in neostriatal structures has also been reported (Austissier et al., 1982; Kontur and Fechter, 1983). Despite the concensus that the central dopaminergic system is disturbed in experimental manganese neurointoxication, the precise details of the temporal, qualitative as well as the quantitative aspects of the disturbances are still controversial.

Recent studies which suggest a relationship between experimental manganese intoxication and some aspect of dopaminergic neurochemistry are reviewed below. These studies are organized in terms of different processes necessary for neurotransmission, namely: 1) synthesis of dopamine and susceptibility of the rate-limiting synthesizing enzyme, tyrosine hydroxylase; 2) release of dopamine into the synaptic cleft and its subsequent inactivation by re-uptake into the nerve terminal; 3) metabolism of the neurotransmitter to inactive products via such enzymes as monoamine oxidase; 4) binding to receptors with consequent biological activity such as changes in ion

channels and increased adenylate cyclase activity. Processes are presented schematically in Figure 6-4.

6.3.2.2.1. Manganese and Synthesis of Dopamine -- Since tyrosine hydroxylase (TOH) catalyses the rate-limiting step in brain catecholamine biosynthesis, the changes in brain dopamine (DA) concentrations in manganese neurotoxicity could simply reflect the changes in the activities of this enzyme. However, there is evidence that the decreased TOH activity, observed ex-vivo in manganese-poisoned animals, cannot be attributed to the direct effect of the metal on this enzyme since Deskin et al. (1981b) did not find any inhibition of TOH activity by 1 mM Mn^{2+}. In young male rats chronically treated with $MnCl_2 \cdot 4H_2O$ (1 mg/mℓ in the drinking water) striatal dopamine level is initially increased and, upon more chronic treatment with this manganese salt, is decreased (Chandra and Shukla, 1981). In adult male rats chronically treated with $MnCl_2$ (10 mg/mℓ in the drinking water) TOH activities in neostriatum, midbrain, hypothalamus and hippocampus, but not in frontal cortex and cerebellum, are increased in the first few months of treatment (Bonilla, 1980). However, upon more chronic treatment with $MnCl_2$, TOH activities are decreased in the neostriatum but its activities in the other brain regions are essentially the same as values in control animals (Bonilla, 1980). Thus in manganese-treated rats the changes in brain TOH activities closely parallel the fluctuations of brain dopamine levels (Bonilla, 1980; Chandra and Shukla, 1981). However, manganese administration by oral gavage in the form of $MnCl_2 \cdot 4H_2O$ at doses of 1, 10 and 20 μg Mn/g bw/day in rat pups during postnatal development for 24 days gives rise to dose-dependent decreases in TOH activities, dopamine levels and dopamine turnover in the hypothalamus (Deskin et al., 1981a). Furthermore, the dose-dependent changes (decreases at the lowest dose but increases

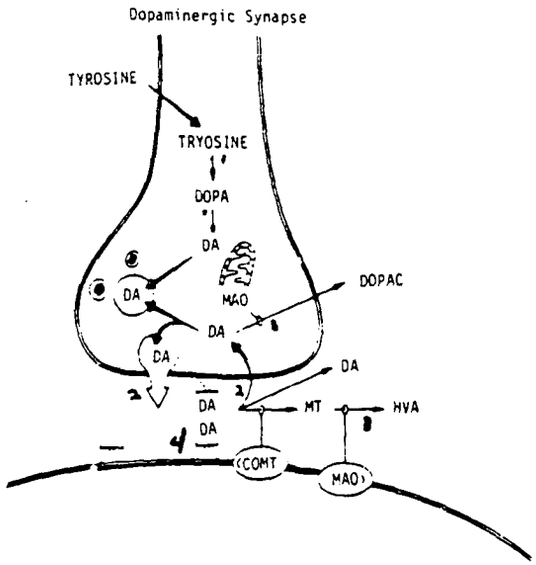

FIGURE 6-4

Schematic representation of a dopamine synapse indicating possible sites of damage produced by manganese exposure: 1) synthesis of dopa by tyrosine hydroxylase, 2) release of dopa and its inactivation by reuptake, 3) dopamine metabolism to inactive products, 4) dopamine bind to post synaptic receptor sites.

Source: Adapted from Cooper et al., 1982

at the higher doses) in tyrosine hydroxylase activities closely parallel the dose-related changes in dopamine levels in the striatum of these manganese-treated rat pups (Deskin et al., 1981a). Employing a different route of administration of manganese (1 mg $MnCl_2 \cdot 4H_2O$ per 100 g/day i.p.), Autissier et al. (1982) also found decreases in striatal dopamine and dopamine turnover in rats 4 months after such treatment.

6.3.2.2.2. Manganese and Dopamine Release and Re-uptake -- Changes in steady-state levels of dopamine can also be accounted for by mechanisms that interfere with the release and re-uptake processes at the nerve endings. For instance, chronic manganese treatment (1 mg $MnCl_2 \cdot 4H_2O$ per mℓ of drinking water) throughout brain development leads to transient, age-dependent but definite decreases in dopamine uptake by synaptosomes, nerve endings containing neurotransmitter storage sites isolated from striatum, hypothalamus or midbrain but not from the cerebral cortex (Lai et al., 1982a, 1983b). These results are compatible with the observations that administration of $MnCl_2 \cdot 4H_2O$ (1 mg/mℓ of drinking water) throughout development gives rise to increased accumulation of this metal in all the brain regions studied, with the exception of the cerebral cortex (Lai et al., 1981b), and that the <u>in vitro</u> inhibitory effects of manganese on dopamine uptake by synaptosomes vary depending on the brain region from which the synaptosomes are isolated (Lai et al., 1981c). However, the <u>in vivo</u> effects of chronic manganese treatment on ex-vivo synaptosomal dopamine uptake vary depending on the dose, since treatment with a higher dose of $MnCl_2 \cdot 4H_2O$ (10 mg/mℓ of drinking water) leads to increased (rather than decreased) synaptosomal dopamine uptake measured ex-vivo (Leung et al., 1982b). In comparison with its effects on synaptosomal dopamine uptake, the effects of manganese on dopamine release have not been extensively studied,

although a recent study by Daniels et al. (1981) reveals that dopamine release by the rat striatal slice preparation is stimulated by 5 μM Mn^{2+}.

6.3.2.2.3. Manganese and Dopamine Metabolism -- Another mechanism by which manganese can influence the steady-state dopamine levels in the brain is through its actions on dopamine metabolism (breakdown). A key enzyme involved in this process is monoamine oxidase (MAO). In earlier studies by Chandra and co-workers (Sitaramayya et al., 1974; Chandra and Shukla, 1978), increased brain activities of MAO in manganese-treated rats were reported. More recently, Chandra and Shukla (1981) found that the striatal MAO activities are only increased during the initial phase of chronic manganese treatment. Others have reported that brain MAO activities in manganese-treated rats show both increases and decreases (Deskin et al., 1981a) or remain unchanged (Kimura et al., 1978; Autissier et al., 1982). However, it is important to point out that, since MAO in brain and other tissues exists in multiple forms (Lai et al., 1980), none of the studies so far discussed (Sitaramayya et al., 1974; Chandra and Shukla, 1978, 1981; Kimura et al., 1978; Deskin et al., 1981a; Autissier et al., 1982) set out to address the effects of manganese on the heterogeneity of MAO. The studies of Lai and co-workers (Leung et al., 1981, 1982a; Lai et al., 1982b; Lai, 1983) were aimed at just trying to resolve the latter question employing specific substrates (serotonin being type A MAO substrate and benzylamine type B MAO substrate) and inhibitors (clorgyline being type A MAO inhibitor and deprenyl type B MAO inhibitor). In rats chronically treated with $MnCl_2 \cdot 4H_2O$ (1 mg/mℓ of drinking water) throughout development until adulthood, only type A MAO activity in the cerebellum is slightly decreased (Leung et al., 1981). In these treated rats, type A MAO activities in all the other brain regions, type B MAO activities in all the brain regions as

well as the type A to type B MAO activity ratios in all the brain regions remain unchanged. Furthermore, the development of type A and type B MAO activities in the whole brain of rats treated with $MnCl_2 \cdot 4H_2O$ (either 1 or 10 mg/mℓ of drinking water) has also been found to be unaltered (Leung et al., 1982a). On the other hand, the same study reveals that both hepatic type A and type B MAO activities in treated animals are increased after 10-15 days of postnatal life. In contrast with the apparent lack of effects of manganese on the A and B forms of brain MAO during development, lifespan treatment of rats with $MnCl_2 \cdot 4H_2O$ (1 mg/mℓ of drinking water for over 2 years) exerts a modulatory effect on the age-related changes of the heterogeneity of brain MAO (Leung et al., 1981). For example, consider the age-related decreases in type A MAO and dopamine-oxidizing activities in striatum and midbrain of manganese-treated rats. In these rats, the other effects are age-related increases in the rates of oxidation of serotonin, benzylamine and dopamine in the cerebellum not observed in control rats (Leung et al., 1981). These results support the hypothesis that chronic manganese encephalopathy may act differentially upon the developing and aging nervous system (Lai et al., 1981a, 1983b; Leung et al., 1981, 1982a; Silbergeld, 1982).

6.3.2.2.4. **Manganese and Effects at the Receptor** -- In human amphetamine addiction, the psychotic behavior closely resembles schizophrenia (Iversen and Iversen, 1975). Neuroleptics that are potent alleviators of the primary symptoms of schizophrenia are good antagonists of CNS dopamine receptors (Iversen and Iversen, 1975). An interesting and enlightening parallel can be drawn between the above two observations and the signs, symptoms and the pathophysiology of chronic human manganism. Since chronic manganese encephalopathy commences with a phase of psychotic behavior

("locura manganica" or "manganese madness") (Cotzias et al., 1971; Barbeau et al., 1976) resembling that of schizophrenia and amphetamine psychosis, and alterations of the central dopaminergic receptor functions have been implicated in the pathophysiology of the latter two syndromes, it is reasonable to hypothesize that one of the neurotoxic mechanisms of manganese may be its effect on these receptors. Several groups of researchers have speculated and proposed that some of the transient neurochemical changes during the initial stages of chronic and very long-term manganese neurointoxication in animals could be viewed as pathophysiological parallels to the initial manifestation of psychotic behavior in human manganism (Bonilla, 1980; Chandra and Shukla, 1981; Lai et al., 1983b,c). There is some evidence that manganese exerts definite effects on the dopamine receptors. The binding of agonist and antagonist to dopamine receptors is potently enhanced by manganous ions (Usdin et al., 1980). Intraperitoneal administration of $MnCl_2$ (10 or 15 mg/kg bw/day) to rats for 15 days results in increased binding of the dopamine antagonist spiroperidol to striatal membranes (Seth et al., 1981). Moreover, manganese also stimulates brain adenylate cyclase activity in vitro (Walton and Baldessarini, 1976). Recently Bonilla (1983) found that striatal adenylate cyclase activity is markedly decreased in rats exposed to 2.5, 5 and 10 mg Mn (as $MnCl_2$) per mℓ of drinking water for 8 months. In addition, the cyclase activity in the treated animals does not respond to stimulation by dopamine (Bonilla, 1983). In rats chronically treated with $MnCl_2 \cdot 4H_2O$ (10 mg/mℓ of drinking water) throughout development, the increases in open-field behavior elicited with i.p. amphetamine administration (1 mg/kg bw) are far less marked (Leung et al., 1982b).

6.3.2.3. IMPLICATIONS OF THE ALTERED METABOLISM OF OTHER NEUROTRANSMITTERS IN MECHANISMS OF MANGANESE NEUROTOXICITY --As noted in the introduction to this chapter and represented in Figure 6-2, dopamine neurons make synaptic contact with neurons located in the neostriatum which contain other neurotransmitters. Some of these neurons are capable of affecting activity in the dopamine-containing cells through a process of feedback inhibition. Thus it is quite possible that toxic damage to these non-dopaminergic cells could in fact have the secondary consequence of altering function in the dopamine neurons. Two nerve types identified in the neostriatum which could serve such a role are those containing the neurotransmitters gamma amino butyric acid (GABA) and those containing acetylcholine. The evidence that manganese may affect these cells is reviewed below.

6.3.2.3.1. GABAergic Systems -- In rats treated with $MnCl_2$ (10 mg/mℓ of drinking water) for 2 months, the caudate gamma amino butyric acid (GABA) level is increased markedly although the activities of glutamic acid decarboxylase (GAD), the rate-limiting enzyme responsible for GABA synthesis, and GABA-transaminase, the enzyme which metabolizes GABA, remain unchanged (Bonilla, 1978a). Employing a developmental rat model of chronic manganese encephalopathy (1 mg $MnCl_2 \cdot 4H_2O$ per mℓ of drinking water throughout development), Lai et al. (1981a) demonstrated that chronic manganese toxicity does not alter the brain regional activities of GAD: these results confirm those obtained by Bonilla (1978a,b) in the rat caudate. Short-term i.p. treatment with Mn (15 mg $MnCl_2$/kg bw/day for 15 days) gives rise to a small decrease in cerebellar GABA binding (Seth et al., 1981).

6.3.2.3.2. Cholinergic System -- Since the pathophysiology of manganese encephalopathy and that of Parkinsonism show certain similarities

(Cotzias et al., 1971) and the cholinergic system may be implicated in the pathogenesis of Parkinsonism (Erickson, 1978), several systematic studies have been initiated to investigate the possibility that the neurotoxic effects of manganese also involve the cholinergic mechanism (Lai et al., 1981a, 1982a,c; Bonilla and Martinez, 1981). In adult rats chronically treated with $MnCl_2 \cdot 4H_2O$ (1 mg/mℓ of drinking water) throughout development, the activities of ChAT, the enzyme that catalyses the synthesis of acetylcholine, decrease slightly in cerebellum and midbrain whereas the activities of this enzyme in the other brain regions as well as the activities of AChE, the enzyme that catalyses the metabolism of acetylcholine, remain unaltered in all the brain regions studied (Lai et al., 1981a). However, in rats treated similarly (Lai et al., 1982a, 1983b), choline uptake by hypothalamic synaptosomes shows an initial decrease (at postnatal ages between 70 and 90 days) and a subsequent increase (at postnatal ages between 100 and 120 days). On the other hand, chronic treatment with two doses of $MnCl_2 \cdot 4H_2O$ (1 and 10 mg/mℓ of drinking water) throughout development does not give rise to any marked changes in the brain regional development of AChE activities (Lai et al., 1982c). Bonilla and Martinez (1981) studied the activities of ChAT and AChE in different brain regions in adult rats treated with 10 mg $MnCl_2$ per mℓ of drinking water for 1-8 months and found virtually no changes in the activities of these enzymes. The results of Bonilla and Matinez (1981) and those of Gianutsos and Murray (1982) are compatible with those of Lai et al. (1981a, 1982c).

6.3.2.3.3. Other Neurotransmitter Systems -- Although the lack of systematic studies precludes any critical and accurate assessment of the possible roles of other neurotransmitters in the pathogenesis and pathophysiology of the neurotoxic effects of manganese, there is some indication that

the noradrenergic system may also be implicated (Chandra et al., 1979c; Chandra and Shukla, 1981; Autissier et al., 1982).

6.3.2.4. MECHANISMS THAT DO NOT DIRECTLY IMPLICATE NEUROTRANSMITTERS -- Recently three other hypotheses have been advanced to account for the possible mechanisms underlying the neurotoxic effects of manganese. Although these hypotheses are presently somewhat speculative in nature, they provide important theoretical frameworks upon which current and future studies are designed.

6.3.2.4.1. Free-radical-mediated Neuronal Degeneration -- This mechanism has been proposed by Donaldson (1981, 1982) to account for neuronal degeneration observed in chronic manganese encephalopathy and other neuronal degenerative diseases. The central theme centers upon the observation that manganese greatly potentiates dopamine autoxidation with the resultant generation of free radicals (e.g., superoxide anion, hydrogen peroxide and hydroxyl radicals) giving rise to degenerative changes (Donaldson, 1981; Donaldson et al., 1981, 1982).

6.3.2.4.2. Autoxidation of Amines to Quinones Enhanced by Manganese -- This hypothesis proposes that increased concentrations of dopamine could result in autoxidation to quinones and liberation of free radicals: both types of reaction products are cytotoxic and could readily give rise to neuronal degeneration (Graham, 1978; Graham et al., 1978). Furthermore, manganese enhances this autoxidation process (Graham, 1983).

6.3.2.4.3. Interactions with Other Essential Metals -- Interactions of manganese with other metals can occur during increased cellular accumulation of manganese in chronic manganese toxicity (Lai et al., 1981b). The increased cellular manganese could either substitute for other metals (particularly divalent metal ions) in their normal capacity (Lai et al., 1983d) or antagonize other metals (e.g., manganese is a potent Ca antagonist).

Under both of these conditions, altered metabolic or cellular regulation may be the predicted result (Lai, 1983; Lai et al., 1983d,e).

6.3.3. Summary. The available results suggest that an accurate dose-response relationship for inhalation exposure and neurotoxicity is unobtainable at present. This is largely due to the fact that criteria for endpoints of effects and routes of adminstering manganese differ in various studies. The single study using inhalation exposure, Ulrich et al. (1979a,b,c), reports no behavioral effects after 9 months exposure to 11.6 $\mu g/m^3$ Mn_3O_4. Unfortunately, this study did not include biochemical data nor levels of manganese in brain tissues. Since there are as yet no good biological indicators of manganese exposure, relating the effects to the tissue levels of manganese would represent a state-of-the-art approach. Despite these shortcomings, evidence is accumulating that one of the key neurotoxic effects of manganese is the disturbance of brain neurotransmitter metabolism.

Chronic exposure of adult rabbits (Mustafa and Chandra, 1972), monkeys (Neff et al., 1969) and rats (Bonilla and Diez-Ewald, 1974) to different manganese compounds gives rise to decreases in brain levels of monoamines, particularly dopamine. More recent studies indicate that chronic treatment of rats with $MnCl_2$ in the drinking water throughout development is associated with selective regional alteration of synaptosomal dopamine uptake but not of serotonin or noradrenaline uptake (Lai et al., 1982b). In the latter studies, the brain regional manganese concentrations show dose-dependent increases (Chan et al., 1981, 1983) and in animals treated with the higher manganese dose, the changes in synaptosomal dopamine uptake is associated with decreased behavioral responses to amphetamine challenge (Leung et al., 1982a). All these observations are consistent with the notion that in chronic manganese toxicity the central dopaminergic system is disturbed.

This hypothesis provides a mechanistic explanation for the extrapyramidal disturbances seen in human manganism.

Results of studies with the rat also strongly suggest that age may play a role in the dose-effect relationship in manganese neurotoxicity (Lai et al., 1981a,b, 1983b; Leung et al., 1981, 1982b). These results suggest that the developing and the aging brain show different susceptibility toward the toxic effects of manganese.

6.4. LUNG EFFECTS

6.4.1. Human Studies. The concept of "manganese pneumonia" (manganese pneumonitis) has been based mainly on epidemiological observations. An association between exposure to manganese and a high rate of pneumonia was first suspected by Brezina (1921), who reported that 5 of 10 workers died of croupous pneumonia within 27 months in an Italian pyrolusite mill. Baader (1932) first ascribed the high incidence of pneumonia among workers making dry cell batteries to manganese. On the basis of his observations as well as upon the reports of Brezina (1921), Schopper (1930), Bubarev (1931), Freise (1933), Dantin Gallego (1935), and Vigliani (1937), Baader (1937) concluded that pneumonia should be regarded as an occupational disease among manganese workers.

Lloyd-Davies (1946) reported the incidence of manganese pneumonia in the manufacture of potassium permanganate. The incidence of this disease among the workers employed over the period 1938-1945 averaged 26 per 1000, compared to an average of 0.73 per 1000 in a control group. All cases were diagnosed as lobar or bronchopneumonia. The impression was that the temperature and general condition of the patient responded more slowly than usual to treatment with sulfonamides. The possible causal relationship to manganese was not suspected until subsequent inquiry. Workers also complained of

symptoms of bronchitis and irritation of the nasopharynx. Manganese concentrations in air, calculated from the MnO_2 content of dust, were between 0.1 and 13.7 mg/m³. Approximately 80% of the particles were <0.2 μ in size and nearly all particles were <1 μ.

Lloyd-Davies and Harding (1949) reported that this high incidence of manganese pneumonitis had been maintained. On the basis of the results of chemotherapy the authors thought it unlikely, with the exception of one case, that bacterial infection played a primary role in producing the consolidation that was unquestionably present in the lung. They concluded that manganese dust in suitable particle size introduced into the respiratory system will, without the presence of other factors, cause pneumonitis.

A high incidence of pneumonia associated with manganese exposure has also been reported by other researchers. Heine (1943) found a high incidence of pneumonia among workers in an alloy producing plant in Aachen, Germany, during the period 1939-1941. However, more careful analysis of the data revealed that during two periods (1936-1938 and 1939-1941) there was no correlation between high incidence of pneumonia and high concentration of manganese in the air in different parts of the factory. Heine concluded that factors other than manganese, such as draft, weather conditions and malnutrition, were predisposing factors for the development of pneumonia.

Rodier (1955) discussed manganese pneumopathies in a study of manganese poisoning in Moroccan miners. Cauvin (1943) had already pointed out the prevalence of pneumonitis associated with the high death rate in miners in Morocco during the winter of 1939-1940 and 1947. Rodier did not consider manganese to be the sole etiological factor, but possibly a factor which aggravated difficulties resulting from the war, poor housing and sanitation.

Problems in obtaining X-ray films and necropsies lead Rodier to conclude it was uncertain whether one was dealing with an ordinary pulmonary infection complication aggravated by manganese, or subacute edema, the pulmonary manifestation of a toxic state.

A higher rate of pneumonia was also reported in basic-slag workers (Jötten et al., 1939). Pneumonia was considered an occupational disease related to the processing, bagging and loading of Thomas slag obtained in the Thomas process of making steel. The Thomas slag contained 6-8% manganese. Baader (1937) assumed manganese and Thomas-slag pneumonia to be similar and the chest symptoms to be caused primarily by the manganese in the slag.

Wassermann and Mihail (1961) studied manganese miners, coal miners and forest workers, all working in comparable geographical areas during the period 1957-1959. The incidence of bronchopneumonia and pneumonia was 26-33/1000 for manganese miners, 0.8-3.0/1000 for coal miners, and 4.8-24/1000 for forest workers. Within each year rates for manganese miners were higher than for other groups. In the manganese mine the concentrations of the dust were 28-840 mg/m^3, and the concentration of manganese ranged from 2-200 mg Mn/m^3 depending on workplace. Particles contained 12-30% manganese and the range of particles <5 μ was 34-81%. Silicon dioxide was also present. Measurements showed manganese concentrations of 55 and 78 mg/m^3 in respiratory zones of workers at two different positions. Radiological examinations showed that 25% of the 820 miners had radiological modifications of varying degrees of severity, characterized by diffuse pulmonary fibrosis and the presence of nodules. Evidence of manganism was reported in 19 workers (2%). Definitive evidence of fibrotic or other specific lung changes has rarely been reported with occupational exposure to manganese aerosols because radiological examinations were not performed.

Fibrotic changes observed by Büttner and Lenz (1937) were almost certainly due to the 20% silica present in dust from the Giessen pyrolusite (MnO_2) mines. Manganoconiosis was confirmed or suspected in 21% of all of the miners and the percentage increased with age and duration of work in the mines.

Van Beukering (1966) performed a study from 1963-1965 in a manganese mine and an iron mine in South Africa and found a pneumonia incidence of 8.08% in over 3000 manganese miners and 5.10% in over 1000 iron miners. No chronic manganism was observed. Saric and Lucic-Palaic (1977) studied three groups of workers to determine whether long-term exposure to manganese may contribute to the development of symptoms of chronic lung disease. The level of manganese exposure was reported as 0.4-16.35 mg/m³ for workers in the production of ferroalloys, 5-40 µg/m³ for workers in the electrode plant and 0.05-0.007 µg/m³ for the workers in the aluminum rolling mill. The latter is low ambient exposure and is considered a control group. The prevalence rate of chronic bronchitis and the respiratory symptoms of phlegm and wheezing was compared in smokers and nonsmokers in the group of ferro-alloy workers and in the control groups. Chronic bronchitis was defined as bringing up phlegm in the morning and during the day and/or night for at least three winter months in the last 2 years or longer. Table 6-9 shows that chronic bronchitis was highest in smokers in the high exposure group. The percentage of chronic bronchitis associated with the objective finding of reduced forced vital capacity was 5% (7/143) in smokers in the alloy plant, greater than in any of the other groups (0 or 1 in each group, hence no statistical testing was appropriate). The rate of respiratory symptoms among smokers did not show an exposure-response association among the group.

TABLE 6-9

Prevalence of Chronic Bronchitis in Groups of Workers According to Smoking Status[a,b]

Exposure to Manganese	Manganese Alloy Production (0.4-16.4 mg/m³)		Electrode plant (5-40 µg/m³)		Aluminum Rolling Mill (0.05-0.07 µg/m³)	
	Number	%	Number	%	Number	%
Smokers	46/143	32.2	14/69	20.3	17/94	18.1
Non-smokers	14/169	8.3	11/102	10.8	4/81	2.0
Total[c]	64/369	17.3	28/190	14.7	25/204	12.3

[a]Adapted from Saric and Lucic-Palaic, 1977

[b]Statistical analysis in original publication is multiple t-tests. This was considered inappropriate and is therefore not presented.

[c]The denominators do not total 369 because data for 57 past smokers are not included.

The tendency for the rate of respiratory symptoms to increase with the extent of the smoking habit was most pronounced in the group of workers in the production of manganese alloys. On the basis of these results, the authors suggest a possible synergism between airborne manganese and smoking habit in the occurrence of respiratory symptoms. However, the results do not support synergism because there is no consistent increase in symptoms among the group. Further, percentages appear to be additive, but data is not sufficient to support this.

Several reports suggest an influence of manganese on the rate of pneumonia and other respiratory ailments among inhabitants living in the vicinity of a ferromanganese factory. In two of these three studies the ambient atmosphere was visibly polluted with dusts, suggesting simultaneous exposure to other contaminants; therefore, effects cannot be definitely attributed to manganese. In 1939, Elstad reported a high rate of lobar pneumonia among the residents of Sauda, a small Norwegian town, after the opening of a manganese ore smelting works in 1923. Data about manganese concentration in air from Sauda are not reliable because only one measurement was made. The report indicates that manganese was contained in visible clouds of brown smoke polluting the atmosphere and the dry matter in the smoke was found to contain silica. From 1924-1935, lobar pneumonia accounted for 3.65% of all deaths in all of Norway and 32.3% of all deaths in Sauda, although the disease had been infrequent in the community until the operation of the plant. Pneumonia attacked inhabitants of the community as well as workers of the plant. Men working at the factory had a 50% higher mortality due to lobar pneumonia than men employed elsewhere. The number of pneumonia cases and deaths varied with the tonnage of manganese alloy produced. The occurrence and types of pneumococci in Sauda did not differ from the rest of Norway.

Nogawa et al. (1973) studied subjective symptoms and ventilatory function in 1258 junior high school students housed in a school 100 m from a ferromanganese plant and in a similar group of 648 students housed 7 km away. These authors cite exposure measures made by the Ishikawa Prefectural Research Institute (White Paper, 1971). Manganese dustfall measured monthly for 3 years averaged 200 kg/km²/month (20,000 ng/cm²/month) in the vicinity of the plant compared to 20-fold lower levels measured at four other points elsewhere in town. In July, 1970, when the survey by Nogawa et al. took place, the manganese concentration in the dust fall was ~100 kg/km²/month. Levels over 200 kg/km²/month did not occur in 1970 until December. Amounts of dustfall and sulfur oxide concentrations plotted over the same time period showed almost no difference between areas within the vicinity of the plant and other areas. Other heavy metals were present but only manganese and iron were high compared to other cities. This dustfall level is indicative of an ambient air concentration of about 3-11 μg/m³, based on similar measurements taken in the vicinity of a U.S. ferromanganese plant (see Table 3-22) where settled manganese dust was related to quarterly measures of airborne manganese. Atmospheric concentration of manganese 100 m from the plant measured by a high volume air sampler was reported as 4.04 μg/m³. The author cites a previous report of a 5-day average of 6.7 μg/m³ at a point 300 m from the plant.

Data on subjective symptoms and medical history of the student and family were obtained in July by 1970 by the British Medical Research Council questionnaire for which the response rate was over 98% in each school. Of the 30 items the following were reported to have higher prevalence in students from the school near the factory: presence of sputum in winter on arising, presence of sputum in summer, wheezing, clogged nose, frequent

colds, and all six items referring to symptoms of the throat. These were reported to be statistically significant at $p<0.05$ but the test used was not specified. The authors addressed several issues which could affect reliability of results. Since ventilation function was related to stature, they compared the stature of students in the two schools and found no difference sufficient to bias results. They noted that the exposure values at the two schools could distinguish among the two groups because students at the polluted school lived within 1500 m of the plant whereas students from the control school lived at least 5 km from the plant. Furthermore, data on schoolchildren are far less likely to be biased by smoking habit and occupational exposure than data on adults. Students from the school near the factory had a higher prevalence of past history of pneumonia. No chronic bronchitis was reported at either school. Objective tests of lung function were measured by the same methods and the same inspectors at both schools with a 97% response rate. Students from the school in the polluted area had lower mean values than students of the control school for forced expiratory volume analyzed by sex and grade. Mean values for the one-second capacity, one-second ratio and maximum expiratory flow were also lower in the school in the polluted area.

In a follow-up study performed after dust collectors had been installed in the factory to reduce the manganese dustfall, the investigators examined respiratory resistance and respiratory symptoms (Kagamimori et al., 1973). The authors concluded that the respiratory symptoms of students in the polluted area improved after manganese exhaust diminished.

In a study on the effect of air polluted with manganese in the vicinity of a plant smelting pig iron and ferromanganese, Dokuchaev and Skvortsova (1962) examined clinical histories of 1200 children up to 16 years of age.

Manganese concentrations in air within a distance ≤1 km from the plant fluctuated from 0.002-0.262 mg/m³. Residents within 0.5 km of the plant complained of visible black dust which accumulated in the homes. Wash water from children's hands contained 38.8 mg Mn/m² of skin area. Managenese was found in 62% of nasal mucosa smears from 700 children. Roentgenological examinations showed pulmonary changes in 75% of the children, many of tuberculous etiology or other residuals of past disease. However, it is not clear how many children were examined nor how incidents were diagnosed or scored. The authors' report of increased inflammatory processes of the respiratory passages due to manganese is not quantitatively supported.

Saric et al. (1975) studied acute respiratory diseases in a town contaminated by a ferromanganese plant. Table 6-10 shows the 3-year cumulative incidence of acute bronchitis (and peribronchitis) in three exposure zones of the town of 31,000 inhabitants. The authors report the differences in the first three rows to be significant but multiple t-tests performed are not appropriate for frequency data and there is no exposure/response effect. The rate of pneumonia in the population of the town did not vary by pollution zone, nor did it show the expected difference between summer and winter periods. Because the concentrations of manganese in the ambient air were higher in summer than in winter, the question was raised whether the expected difference was masked by respiratory disease associated with observed seasonal variations in the level of manganese. Incidence rates were presented by age, but rates by zone were not age-adjusted. Locations of the workers' homes were not given but workers represented only a small percent of the population since only 100 lived in the town (Saric, 1983). The authors also stressed the fact that in this study some other potentially relevant factors may not have been sufficiently controlled.

TABLE 6-10

Cumulative Incidence of Acute Respiratory Diseases During the 3-Year Period*

Mn Concentration ($\mu g/m^3$)	0.27-0.44 I (N=8690)		0.18-0.25 II (N=17105)		0.05-0.07 III (N=5296)	
	Number	%	Number	%	Number	%
Acute bronchitis and peribronchitis						
Winter	474	5.5	1125	6.6	2261	4.3
Summer	296	3.4	698	4.1	141	2.7
Pneumonia						
Winter	47	0.5	84	0.5	17	0.3
Summer	39	0.4	93	0.5	19	0.4

*Adapted from Saric et al., 1975

6.4.1.1. SUMMARY -- The studies of occupational exposures support the association of pulmonary effects and exposure to manganese. Most of these exposures range higher than the present limit in the United States for occupational exposure, 5 mg Mn/m³, so they provide little information on the possible effects of exposures to ambient levels. These studies were examined to determine if exposure levels could be associated with a severity of respiratory effects. However, conclusions about these exposure/response relationships are limited because exposure values often cover a broad range and pulmonary endpoints may not be clearly described or vary among studies. The health effects of simultaneous exposures have also not been thoroughly examined; for example, exposure to silica may account for some of the more dramatic increases in pneumonia.

Table 6-11 summarizes those studies which report levels of exposure to manganese. The study in schoolchildren (Nogawa et al., 1973) was sufficiently well documented to support an association between the increased respiratory symptoms in children and exposure to the dusts containing manganese from the emissions of the ferromanganese plant estimated by EPA to correspond to exposure levels of 3-11 μg/m³. It is plausible that exposure to manganese may increase susceptibility to pulmonary disease by disturbing the normal mechanism of lung clearance. Uncertainties regarding manganese as an etiological factor in the development of pulmonary diseases (i.e., pneumonia) among workers prompted the animal studies described in the next section.

6.4.2. Animal Studies. Studies with animals (Table 6-12) have helped clarify the effect of manganese on the lungs. These findings suggest that a primary inflammatory reaction of limited duration, without the presence of pathogenic bacteria, may occur in the lung after exposure to manganese.

TABLE 6-11

Summary of Human Studies of Respiratory Effects at Various Levels of Exposure to Manganese

Type of Exposure	Exposure Level	Chemical/ Particle Size	Number	Response	Reference
Manganese miners (Roumania)	2-220 mg/m³	34-81% smaller than 5 μ -- various work-places. Some SiO_2.	820	Increased frequency of pneumonia compared to coal miners and forest workers. Radiological modifications in lungs of 25% of miners. 2% manganism.	Wassermann and Mihail, 1961
Manufacture of potassium permanganate; workers (England)	0.1-13.7 mg/m³	MnO_2/80% <2 μ	NR	Increased incidence of "pneumonia" in workers averaged 26 vs. 0.73/1000 in controls. Increased frequency of bronchitis.	Lloyd-Davies, 1946
Manganese alloy workers and worker controls (Yugoslavia)	I 0.4-16.0 mg/m³ II 5-40 μg/m³ III 0.05-0.07 μg/m³	NR NR NR	369 190 204	Increased prevalence of chronic bronchitis in group I. Particularly in smokers. Respiratory symptoms did not vary with exposure to manganese	Saric and Lucic-Palaic, 1977
Emissions from ferromanganese plant; school-children (Japan)	3-11 μg/m³* vs. 10 to 45-fold lower	NR	1,235 640	Increased prevalence of respiratory symptoms (e.g., sputum, wheezing, sore throat). Lower mean values in objective tests of lung function. No chronic bronchitis in either school.	Nogawa et al., 1973
Emissions from manganese alloy plant; town residents (Yugoslavia)	0.27-0.44 μg/m³ 0.18-0.25 μg/m³ 0.05-0.07 μg/m³	NR NR NR	8,690 17,105 5,296	Incidence rate of acute bronchitis higher in zone II: inconclusive.	Saric et al., 1975

*Estimated by EPA

All workers were males; race not given.

NR = Not reported

TABLE 6-12

Respiratory Effects with Manganese Exposure:
Intratracheal, Intraperitoneal and High Dose Inhalation Exposures

Species	Compound	Concentration	Exposure	Effects by Exposure Type: Mn Only	Mn + Bacteria	Bacteria Only	Results	Reference
Rabbit	MnO_2	NR	1 hour/day/ 39+20 day	+	+	-	Rabbits infected with pneumococci after 39 days. Bronchial lesions with leukocyte and lymphocyte infiltration noted histologically in Mn and Mn + Bacteria groups. Highest mortality in Mn + Bacteria group. No pathological changes in Bacteria group.	Jotten et al., 1939
Mice	MnO_2	NR	1 hour/day/29 day	-	+	+	More severe bronchopneumonic changes observed in mice that were first immunized with killed pneumococci than in mice exposed to MnO_2 plus viable pneumococci without prior immunization. No bronchopneumonic changes in Mn only group.	Jotten et al., 1939
Guinea pig	ferro-manganese	2350 mg/m³	8 hours/day/ 6 months	-	NS	NS	No histopathological changes in lungs.	Heine, 1943
Guinea pig	ferro-manganese	2350 mg/m³	8 hours/day/ 7.5 months	-	+	NS	Guinea pigs infected with pneumococci. Mortality rate similar in unexposed, unexposed plus immunized, exposed, and exposed plus immunized.	Heine, 1943
Mice	ferro-manganese	NR	15 minutes/day/ 31-102 days	-	+	+	No significant difference in mortality rate (~100%) between infected unexposed and infected exposed mice. Mortality rates were 30-40% lower among immunized than among nonimmunized animals.	Heine, 1943
Mice	MnO_2	NR	2.70 or 120 minutes/ day/15-21 days	-	+	+	Mice infected with pneumococci and/ or *Streptococcus hemolyticus*. Histological changes (slight to intense mononuclear interstitial infiltration of bronchi, bronchial and alveolar epithelium edema) depended on length of exposure. Exposed animals did not show increased susceptibility to pneumococcus.	Lloyd-Davies, 1946

TABLE 6-12 (cont.)

Species	Compound	Concentration	Exposure	Effects by Exposure Type: Mn Only	Mn + Bacteria	Bacteria Only	Results	Reference
Rat	MnO_2 solution	10 mg	single intra tracheal	+	NS	NS	Rats killed at intervals from 1 hour to 18 months postinjection. Inflammatory response within 15 minutes (bronchiolarepithelial changes) to 24 hours (mononuclear reaction in the interstitial tissue). Subsequently, widespread pneumonia and a granulomatous reaction sometimes developed.	Lloyd-Davies and Harding, 1949
Rat	$MnCl_2$	50 mg	single intra-tracheal	+	NS	NS	Rats killed at intervals from several minutes to 8 days postinjection. Death due to gross pulmonary edema within a few minutes, but lung histology normal.	Lloyd-Davies and Harding, 1949
Rat	$MnCl_2$	5 mg	single intra-tracheal	+	NS	NS	Rats killed as above (50 mg). Death due to pulmonary edema in 1/3 of the rats within 1 hour. Alveoli normal in survivors after 1 week, but mucoal cells in the bronchial epithelium remained abnormal.	Lloyd-Davies and Harding, 1949
Guinea pig	MnO_2	50 mg	intratracheal	+	++	+	Simultaneous inoculation of Mn and Candida albicans. Inflammatory reaction developed earlier, was more intense and widespread, and eventually produced more fibrosis (>120 days) in the Mn + Bacteria group.	Zaldi et al., 1973
Monkey	MnO_2	3 mg/m³	22 hours/day/ 5 months	+	NS	NS	Pulmonary congestion.	Nishiyama et al., 1975
Monkey	MnO_2	0.7 mg/m³	22 hours/day/ 5 months	+	NS	NS	Pulmonary changes were less severe and appeared later than in 3 mg/m³ exposure.	Nishiyama et al., 1975
Mice	MnO_2	3 mg/m³	22 hours/day/ 2 weeks	+	NS	NS	Inflammatory changes were generally reversible after 7 months, at which time desquamation of the bronchial epithelium was observed.	Nishiyama et al., 1975
Mice	MnO_2	0.7 mg/m³	22 hours/day/ 2 weeks	+	NS	NS	Same pulmonary changes as in the 3 mg/m³ mice.	Nishiyama et al., 1975

TABLE 6-12 (cont.)

Species	Compound	Concentration	Exposure	Effects by Exposure Type: Mn Only	Mn + Bacteria	Bacteria Only	Results	Reference
Guinea pig	MnO_2	50 mg	single intratracheal	+	NS	NS	Mn particles were not phagocytized until 30 days postinjection. Degenerative changes in the macrophages, infiltration of eosinophils, and some reticulitis at >90 days.	Shanker et al., 1976
Mice	MnO_2	109 mg/m³	1-3 hours/day/ 1-4 days	+	++	+	Mice infected with Klebsiella pneumonia within 1-5 hours after final 3-hour exposure. Inflammatory response was most marked after the 4th exposure to Mn only, but no deaths. Mortality was 2-fold higher in the Mn + Bacteria group than in the bacteria only group, and mortality was greatest in animals with longest intervals between MnO_2 exposure and bacterial challenge.	Kalgetter et al., 1978
Rat	MnO_2	50 mg	intratracheal	-	NS	NS	Most of the animals showed normal pulmonary histology after 30 days, although some had nodules of dust, macrophages and thin reticulin fiber. Enzyme activities from lung fractions normal.	Singh et al., 1977
Guinea pig	MnO_2	22 mg/m³	24 hours	+	++		Initial depression of number and phagocytic capacity of macrophages and bacterial clearance mechanism, but nearly all MnO_2 was cleared from the lungs by 7 days postexposure. The inflammatory reaction was more pronounced in the lungs challenged with bacteria, but tissue histology was normal.	Bergström, 1977

NS - Not studied; NR - Not reported

Histological examination of lung tissue from animals exposed to manganese by inhalation indicates that slight to intense leukocyte infiltration characterizes the acute pulmonary responses (Jötten et al., 1939; Lloyd-Davies, 1946; Malgetter et al., 1976; Bergström, 1977). Since other clear-cut histological findings have not been observed in acute manganese exposure, Bergström (1977) suggests that the acute pulmonary effects may have been overlooked in the early inhalation studies based only on histological evaluation (Heine, 1943). Bergström (1977) also notes that the occurrence of a primary inflammatory reaction is consistent with the ineffectiveness of usual antibiotics used for pneumonia treatment in the acute phase after MnO_2 exposure.

The available experimental evidence indicates that it is unlikely that exposure to manganese could be solely responsible for the development of the serious pathological changes in the lungs (e.g., bronchopneumonia or pneumonitis, chronic inflammatory effects such as fibrosis); instead, it is likely that susceptibility to infection is increased. Table 6-12 shows the pulmonary effects of exposure to manganese with and without simultaneous exposure to bacteria. Since the pulmonary reaction after exposure to manganese is more pronounced in lungs challenged with bacteria (Jötten et al., 1939; Heine, 1943; Lloyd-Davies, 1946; Zaidi et al., 1973; Malgetter et al., 1976; Bergström, 1977), and because sufficient evidence indicates that exposure to manganese has a depressive effect on the number and phagocytic capacity of alveolar macrophages (Waters et al., 1975; Graham et al., 1975; Shanker et al., 1976; Bergström, 1977), the serious pathological changes should probably be attributed to decreased resistance to respiratory infection and the presence of pathogenic bacteria. The results of the study by Jötten et al. (1939) with immunized mice further suggest that manganese may interfere with

some immunological mechanism, rendering the animals more susceptible to infections. Consequently, some of the earlier observed cases of manganese pneumonia might have had a bacterial genesis, particularly among population groups whose exposure to manganese was low and risk of airborne infection was high.

The experimental data on the pulmonary toxicity of manganese in Table 6-12 consists of studies at high doses of short duration and often using intratracheal administration, therefore not showing a consistent dose-response relationship. For example, Singh et al. (1977) reported that an intratracheal inoculation of 50 mg MnO_2 did not produce significant biochemical or pathohistological changes in the lung tissue, although one of the authors observed in his earlier investigations that MnO_2 alone produced fibrotic reaction under exactly the same experimental conditions (Zaidi et al., 1973; Shanker et al., 1976). In other studies pathomorphological changes were observed in the lung tissue of experimental animals after intratracheal inoculation of 10 mg MnO_2 (Lloyd-Davies and Harding, 1949; Levina and Robacevskaja, 1955), and even after 5 mg $MnCl_2$ (Lloyd-Davies and Harding, 1949). However, it is reasonable to conclude that the usually rapid lung clearance of inhaled manganese (Maigetter et al., 1976; Bergström, 1977) is ineffective in the intratracheal inoculation, so that an amount of 5 mg manganese is sufficient to induce local lesions in the lung.

Although inhalation studies represent a much better experimental model for studying pulmonary effects, the results obtained are still insufficient for estimating accurate dose-response relationships for inhalation exposure to manganese. For example, Heine (1943) found no pathological changes in the lungs of guinea pigs exposed to 2350 mg/m³ ferromanganese dust 8 hours/day for up to 200 days. Further, in experiments on rats exposed to 150 mg MnO_2/m³ for up to 15 months, no signs of pneumonia were observed.

Table 6-13 contains more recent inhalation studies administering lower doses and using longer time periods; hence, these studies are more useful for delineating effects at level near ambient exposures.

A series of inhalation studies reporting acute exposures to Mn_3O_4 aerosol are supportive of the pulmonary toxicity of manganese (Adkins et al., 1980a,b,c). Charles River CD-1 mice (4-8/group) were exposed for 2 hours to Mn_3O_4 aerosol in concentrations ranging from 0-2.9 mg Mn/m³. Dry/wet ratios of tissue weight were examined as an index of edema and the results were not considered to be biologically significant (Adkins et al., 1980a). Another experiment was designed to examine the suppression of pulmonary defense mechanisms after acute inhalation exposure to manganese. Exposure of groups of 22-195 mice for 2 hours to 897 µg Mn/m³ significantly reduced the total number of microcytic pulmonary cells ($p<0.01$, t-test), but did not affect the differential cell count (macrophages, PMNs, lymphocytes). Reduction in phagocytic capability was not statistically significant (Adkins et al., 1980b).

Adkins et al. 1980c also exposed 20-80 mice/group to Mn_3O_4 (0.22-2.65 mg/m³) and subsequently to airborne Streptococcus pyogenes. Animals exposed to manganese showed higher mortality rates than infected control animals (at 0.38 mg Mn/m³ a 7.5% mortality increase was within 95% confidence limits). These results support the concept that a primary inflammatory reaction to manganese can occur in the respiratory tract after exposure to manganese, causing a decrease in the resistance to respiratory infections.

Ulrich et al. (1979a,b,c) exposed Sprague-Dawley rats (30/group) and squirrel monkeys (8/group) to Mn_3O_4 aerosol at concentrations of 11.6, 112.5 and 1152 µg Mn/m³ for 24 hours/day, 7 days/week for 9 months.

TABLE 6-13

Respiratory Effects with Manganese Exposure: Inhalation Exposures at Low Doses

Species	Compound	Concentration (particle size)	Exposure	Effects: Mn Only	Effects: Mn + Bacteria	Effects: Bacteria Only	Comments	Reference
Mice, Charles River, CD-1 (20-195/group)	Mn_3O_4 aerosol	897 µg/m³ (1-3 µm)	2 hours	-	NS	NS	Normal cell concentration (macrophages, PMNs, lymphocytes). No increase in extracellular protein (no edema). No effect on phagocytic capability.	Adkins et al., 1980b
Mice, Charles River, CD-1 (20-41/group)	Mn_3O_4 aerosol	220-2650 µg/m³ (1-3 µm)	2 hours	NS	++	+	(Bacteria only = control) Mean mortality rate increases over controls as Mn concentration increases. Enhanced growth of streptococci over controls.	Adkins et al., 1980c
Rats, Sprague-Dawley (30/group) Monkeys, squirrel (8/group)	Mn_3O_4 particulate	Control 11.6 µg 112.5 µg 1152 µg (<2 µm)	24 hours/day, 9 months	-	NS	NS	No exposure related gross or microscopic alterations or effects on mechanical or ventilatory properties of the lung. (No exposure related effect on EMG or limb tremor.	Ulrich et al., 1979a,b,c
Monkeys, Rhesus (7 exposed, 5 controls)	Mn_3O_4 particulate	100 µg/m³	24 hours/day to 66 weeks	-	NS	NS	No abnormal changes seen on gross or microscopic examinations. Increase of Mn in lung. 8/12 had acariasis.	Coulston and Griffin, 1977
Monkey, Rhesus A) 3 exposed B) 2 exposed	MnO_2 dust	A) 3000 µg/m³ B) 700 µg/m³	22 hours/day, 10 months	+	NS	NS	Inflammatory changes earlier in A than B; granular rather than infiltrative shadows. After 10 months hyperplasia of lymphoid tissue, pulmonary emphysema. Deposits of dust in macrophages.	Suzuki et al., 1978
Rats (74/group) Hamsters, golden, (60/group)	Automotive emissions containing Mn	117-131 µg/m³ (0.3 µm) plus other particulate and gases	8 hours/day, 56 days	-	NS	NS	No gross or microscopic changes in the lung.	Moore et al., 1975

NS = Not studied

Although blood and tissue levels of manganese were elevated in both species at the high dose after 9 months, significant exposure-related effects were not reported in either species after neurologic, histopathologic, organ weight, pulmonary function or hematologic observations. The investigators evaluated pulmonary physiology data for the 4 exposure groups of monkeys each at 5 points in time but the report presents only the mean percent of pre-exposure values in groups of 4 after 9 months of exposure (Table 6-14). Few statistically significant differences were found using the Mann-Whitney U test. Mean value showed increased airway resistance in some of the exposed groups and standard error of the mean showed wide intra-group variability. The authors conclude that there were no time-related effects or trends attributable to manganese exposure. However, it is not clear which two groups were compared statistically, which is particularly confusing since there are four groups in the experiment. Data over time is not presented, regression methods are not used, and numbers of animals tested are too small to detect lung damage unless it is quite severe. Furthermore, a 9-month exposure period even at 24 hours would not qualify as a chronic study in the monkey and thus might be inadequate for the development of detectable lung damage at these exposure levels.

The authors state that lungs were free of inflammatory and/or degenerative changes. The microscopic examination is not described. Thus this study as reported does not present sufficient evidence for lack of adverse pulmonary effects because of small sample size within group variability, insufficient exposure duration and inadequate statistical analysis. It does support a lack of gross toxic effects at this level. Serum biochemical evaluations showed some evidence of hypophosphatemia in the male rats

TABLE 6-14

Pulmonary Physiology Data for Male and Female Monkeys After Nine Months of Exposure[a]

Evaluation	Group	Mean Percent Of Pre-Exposure Values	
		Males (n=4)	Females (n=4)
		Mean ± SEM[b]	Mean ± SEM
Respiratory rate	I control	134 ± 31	150 ± 14
	II 11.6 µg	88 ± 9	106 ± 10
	III 11.25 µg	175 ± 32	149 ± 9
	IV 1152 µg	157 ± 32	143 ± 14
V_T (tidal volume)	I	90 ± 4	115 ± 15
	II	141 ± 33[c]	104 ± 14
	III	98 ± 16	185 ± 36
	IV	94 ± 11	100 ± 24
MV	I	122 ± 30	205 ± 30
	II	124 ± 29	116 ± 19
	III	160 ± 22	272 ± 46
	IV	143 ± 29	136 ± 27
R (pulmonary flow resistance)	I	209 ± 125	80 ± 35
	II	104 ± 9	204 ± 45
	III	365 ± 83	99 ± 39
	IV	257 ± 124	308 ± 272
Cdyn (dynamic compliance)	I	91 ± 33	106 ± 23
	II	125 ± 45	74 ± 13
	III	54 ± 10	153 ± 43
	IV	123 ± 23	92 ± 33
N (1% N_2)	I	103 ± 12	192 ± 20
	II	116 ± 23	95 ± 10
	III	105 ± 2	156 ± 28
	IV	74 ± 7	73 ± 11

[a]Adapted from Ulrich et al., 1979c

[b]The authors state that the Mann-Whitney U was used for statistical comparisons, and the standard error of the mean is presented to provide some index of the variability.

[c]$p = 0.028$

exposed to 1152 μg Mn/m³, but the toxicologic significance of this finding is uncertain. The amount of manganese present in the diets of the animals was not stated.

Coulston and Griffin (1977) exposed seven rhesus monkeys to 100 μg Mn/m³ as particulate Mn_3O_4 due to combustion of MMT for 6, 12 or 15 months. The conclusion states that there were no abnormalities on gross or microscopic examinations. However, no objective measures of pulmonary function were reported. Peribronchiolitis and pneumonitis was reported in association with infection to mites (acariasis) in 6 of 7 exposed monkeys, and a statistically significant increase in manganese in the lungs was reported in 2 controls.

Moore et al. (1975) studied chronic exposure to automobile emissions from the combustion of gasoline with MMT additive. The average concentration was 117 μg Mn/m³ over 56 days, 8 hours/day. No gross or microscopic changes were seen in lungs of exposed animals. [For a review of the toxicology of MMT see Stara et al. (1973).]

6.4.2.1. SUMMARY -- Information from earlier studies on the pulmonary toxicity of manganese is incomplete and sometimes contradictory, particularly in respect to the exposure-response relationship. Some pathomorphological changes in the lung tissue of experimental animals were observed after intratracheal inoculation of 10 mg MnO_2 or after 5 mg $MnCl_2$ (Lloyd-Davies and Harding, 1949).

Inhalation studies represent much better experimental models for studying pulmonary effects. Experimental evidence indicate that acute respiratory effects appear when the level of exposure exceeds 20 mg/m³ of MnO_2 (Bergström, 1977; Maigetter et al., 1976). Although studies of toxicity

after chronic exposure have deficiencies which limit their use for delineating exposure-response levels, several studies exist in which experimental animals were exposed to MnO as Mn_3O_4 particle or aerosols of respirable particle size, an appropriate form for health risk evaluation for airborne manganese. Suzuki et al. (1978) reports positive radiologic findings after 10 months of exposure to manganese dioxide dust at higher levels, 0.7 mg/m³, and Adkins et al. (1980c) report increased mortality from infection in mice at ~0.4 mg/m³.

Table 6-13 shows the two studies in which the lowest levels of exposure to manganese occurred (Ulrich et al., 1979a,b,c; Coulston and Griffin, 1977). These report no effect due to the exposure, but the latter, in particular, had deficiencies which reduce confidence in the negative results. The existence of three negative studies in this range supports a lack of gross toxic effect at this level.

6.5. REPRODUCTIVE EFFECTS

6.5.1. Human Studies. Impaired sexual behavior in workers showing symptoms of manganism has often been reported. Diminished libido or impotence have been the most common symptoms (Penalver, 1955; Mena et al., 1967; Emara et al., 1971; Chandra et al., 1974; Cook et al., 1974). Rodier (1955) reported impotence in ~80% of his patients, although this symptom can be preceded by a short phase of sexual stimulation. Emara et al. (1971) reported one case of hypersexuality which was not followed by diminished libido.

6.5.2. Animal Studies. Influence of manganese exposure on sexual behavior in experimental animals has not been reported in the literature. However, studies have been done on histological, biochemical and/or morphological changes. Chandra (1971) reported that i.p. administered $MnCl_2$ (8 mg/kg bw daily) in rats caused no histological changes in seminiferous

tubules for up to 30 days of exposure. Marked degenerative changes in these tubules did occur after 150 and 180 days of exposure. The affected tubules (~50%) showed marked depletion or absence of spermatids and spermatocytes and a number of degenerated spermatogenic cells. Chandra and colleagues initiated a series of experiments in rats injected i.p. with 6 mg Mn/kg bw daily (as $MnSO_4 \cdot 4H_2O$) in order to elucidate the mechanism of testicular damage (Singh et al., 1974, 1975; Tandon et al., 1975; Chandra et al., 1975). The exposure periods were from 25-30 days. The number of tubules showing degenerative changes was less (~10%) than in the study by Chandra (1971). The rest of the tubules and interstitial tissue showed no morphological changes. Degenerative changes were accompanied by a decrease in the activity of some enzymes, such as succinic and lactic dehydrogenases (SDH and LDH), and acid phosphatase (AP), and an increase in manganese concentration in the testes. The authors explained their histological findings as manganese-induced inhibition of enzymes involved in energy metabolism of the cells. Simultaneous administration of zinc had a beneficial effect, but various chelating agents failed to improve morphological changes.

In another experiment in rats, Shukla and Chandra (1977) administered $MnCl_2 \cdot 4H_2O$ i.p. (15 mg/kg bw daily) for 15, 30 or 45 days. An increase in manganese concentration in brain, liver and testes was accompanied by a decrease in nonprotein sulfhydryls, and a reduction in activity of glucose-6-phosphate dehydrogenase and glutathione reductase. This was explained by possible reduction of cysteine content of the tissues due to formation of manganese-cysteine complex and its excretion from the body. Oral administration of $MnCl_2 \cdot 4H_2O$ (50 µg/kg bw daily) to rats for 180 days did not induce chromosomal damage in the bone marrow or spermatogonial cells (Dikshith and Chandra, 1978). In this experiment, however, the

daily oral dose was at least 500 times lower than the recommended daily dietary intake of manganese. This makes it very difficult to evaluate these data.

In rabbits a single intratracheal injection of MnO_2 (250 mg/kg bw, particle size <5 μm) resulted in marked destruction and calcification of the seminiferous tubules at 8 months after exposure (Chandra et al., 1973a). There was extensive desquamation and cytolysis of various elements of the epithelium with markedly degenerated spermatocytes and spermatids. Females kept with experimental males did not become pregnant, but no details on the reproductive performance testing procedure were given. Similar to results observed in rat experiments, the activities of some enzymes were significantly reduced (ATPase, SDH and AP). Seth et al. (1973) using the same experimental design in rabbits, showed that degenerative changes in ~10-20% of seminiferous tubules were present at 2 months after exposure and gradually increased showing severe changes at 8 months.

In an attempt to investigate whether the early histochemical effects of manganese on testicular enzymes occur prior to morphological changes, Imam and Chandra (1975) administered $MnCl_2 \cdot 4H_2O$ i.v. to rabbits (3.5 mg/kg bw daily) for up to 30 days. Manganese inhibited SDH activity in seminiferous tubules 5 days after the beginning of exposure, when morphological alterations were not apparent. They demonstrated that manganese affects the germinal function of testicular tissue without disturbing steroidogenesis, and reached the same conclusion on manganese-induced disturbances in energy metabolism as in rat experiments.

[illegible] and Ahlström (1975) exposed female rats to manganese in diet [illegible] 8 weeks and during pregnancy. $MnSO_4 \cdot 7H_2O$ was the dietary [illegible] final manganese [illegible] in the diet were 4, 24,

54, 154, 504 and 1004 mg Mn/kg. Exposed animals had normal reproductive performance. No gross malformations or bone structure anomalies were observed in the fetuses. Body weights, dry matter and ash contents were also not affected by dietary exposure to manganese of their dams. At higher manganese levels (104, 504 and 1004 ppm) there was an increase in whole body content of manganese in fetuses as well as in livers of their dams, but no increase in liver manganese was found in nonpregnant females.

Epstein et al. (1972) used a modified dominant lethal assay for 174 test agents. $MnCl_2$ was injected i.p. to male ICR/Ha Swiss mice (20 or 100 mg/kg bw). Animals were mated for eight consecutive weeks and the authors classified $MnCl_2$ as an agent producing early fetal deaths and preimplantation losses within control limits. In a similar study using dominant lethal test procedures Jorgenson et al. (1978) administered $MnSO_4$ to male rats by single or multiple gavages at three dosage levels (levels not mentioned), and concluded that $MnSO_4$ was not mutagenic to the rat.

Gray and Laskey (1980) investigated the reproductive development associated with chronic dietary exposure to manganese. Male mice (CD-1) were exposed to 1050 ppm Mn as Mn_3O_4 in a casein diet from day 15 of lactation to 90 days of age. Wet weights of preputial glands, seminal vesicles and testes measured at 58, 73 and 90 days of age were lower in exposed than in control animals. Body and [illegible] weights were not affected. Reproductive performance was not [illegible]

Laskey et al. (1982) designed a follow-up study in rats to evaluate the effects of dietary manganese [illegible] on reproductive development. Lo[illegible] on day 2 of mothers' gesta[illegible] 1050 and 3500 ppm [illegible]ese added [illegible]e 1.02 μm) to a normal

ATTACHMENT B-2

POTENTIAL HEALTH EFFECTS OF MANGANESE IN EMISSIONS FROM TRAP-EQUIPPED DIESEL VEHICLES

A REPORT FROM THE HEALTH EFFECTS INSTITUTE
SEPTEMBER 1988

HEI

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POTENTIAL HEALTH EFFECTS OF MANGANESE IN EMISSIONS FROM TRAP-EQUIPPED DIESEL VEHICLES

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(240 µg Fe/g; 50 µg Mn/g) and an iron-deficient (20 µg Fe/g; 50 µg Mn/g) diet. Testes weights were not affected, but of particular interest was the manganese dose-related decrease in serum testosterone concentration without a concomitant increase in serum LH concentration. Fertility, measured as percent pregnant, was reduced in females at 3500 ppm (females were mated with males from the same dosage group). Although this difference was statistically significant compared to controls, all other reproductive parameters (litter size, number of ovulations, resorptions and preimplantation deaths, as well as fetal weights) were within control values in all manganese-treated groups.

6.5.3. Summary. Except for reports of impotence in patients with chronic manganese poisoning, human data are largely lacking.

Existing animal data are most concerned with possible reproductive failure in males. Chandra and co-workers suggested that the changes in testes occur prior to changes in brain. However, with the exception of one study on rabbits (Chandra et al., 1973a), reproductive performance was not tested. These results, however, were obtained using parenteral routes of exposure, thus being of limited value in predicting reproductive hazards of ingested or inhaled manganese.

The few remaining studies are not in agreement with the Chandra studies. They show that manganese is not likely to influence reproductive parameters. The most accurate studies describing long-term dietary exposure to manganese show that dietary levels up to 1004 ppm (Järvinen and Ahlström, 1975) as $MnSO_4 \cdot 7H_2O$ and up to 3550 ppm (Laskey et al., 1982) as Mn_3O_4 were almost without effect on reproductive performance. However, some observations in all these studies need to be verified using well-defined reproductive testing protocols.

6.6. HEMATOLOGIC EFFECTS

6.6.1. Human Studies. Reports about the effect of manganese on human blood and hemoglobin show conflicting results. The studies are difficult to compare because of variations in exposure and stage of disease or effect. Kesic and Häusler (1954) reviewed these data and suggested that many authors had not considered the variability in normal individuals.

Kesic and Häusler (1954) reported hematological data comparing 52 exposed miners without symptoms of poisoning to 60 sawmill workers of similar age and social conditions. The miners had higher mean levels of erythrocytes, 4.5×10^{-6} compared to 4.3×10^{-6}. Mean hemoglobin levels were higher in miners, 15.03 compared to 14.19 g, and mean monocyte levels were lower (6.4 vs. 7.8%).

In a study on industrial manganese poisoning, Flinn et al. (1941) found a low white cell count in a group of 23 workers exposed to manganese. The average white cell count was 5380 for the workers as compared to 7850 and 7560 for the two control groups. Seven of the 11 affected men had a white cell count <5000 and, of these, three men had a count <4000. In general, leucopenia became more pronounced with the progress of the disease.

Chandra et al. (1974) reported lower erythrocyte counts (RBCs) and lower hemoglobin concentrations in 12 cases diagnosed as manganese poisoning compared to 20 controls. Both cases and controls were under age 38; 3 cases were mild, 8 moderate, and 1 severe according to the system of Rodier (1955). The RBC levels ranged from $3.5\text{-}4.8 \times 10^{-6}/mm^3$, and controls from $5.0\text{-}5.6 \times 10^{-6}/mm^3$. Hemoglobin levels for cases and controls were 11-14.5 g/100 ml and 15-17 g/100 ml, respectively. Total white blood cell counts ranged from 7000-11,000/mm^3 in both groups with a normal percentage of white cell forms.

Paterni (1954) claimed that small doses of manganese had a stimulatory effect on erythropoiesis. From other findings encountered in chronic manganese poisoning, it was presumed that large amounts of manganese caused depression of both erythropoiesis and granulocyte formation (Cotzias, 1958). Rodier (1955) also reported a change in white-cell count in 52% of patients with manganism, with a relative increase of lymphocytes and a decrease in the number of polymorphonuclear cells. Details and a comparison group are lacking.

6.6.2. Animal Studies. Animal studies have confirmed some of the observed hematological effects in humans. For example, Baxter et al. (1965) found that hematocrit and mean corpuscular volume were significantly increased in rats receiving 150 mg Mn/kg bw s.c., while serum calcium and iron were markedly depressed. Blood volume was unchanged; serum magnesium, chloride, and phosphorus showed significant increases. Similar findings were reported by Doi (1959), who exposed rabbits to MnO_2 in specially designed inhalation chambers. Both erythrocyte count and hemoglobin content tended to increase. The leukocyte count changed more extensively with a relative increase of lymphocytes. Matrone et al. (1959) found that 2000 ppm of manganese in the diet depressed hemoglobin formation in both rabbits and baby pigs. They estimated that the minimal level of manganese in the diet that interfered with hemoglobin formation was between 50 and 125 ppm. Similarly, Hartman et al. (1955) showed that 2000 ppm of manganese in the diet interfered with hemoglobin regeneration in lambs.

Carter et al. (1980) exposed two groups of Long-Evans rats to four levels of manganese as Mn_3O_4 at 50 ppm (normal dietary level), 400, 1100 and 3550 ppm. One group was maintained on a normal diet, the other on an iron-deficient diet. After exposure to Mn_3O_4 during the prenatal and

postnatal period, no changes in red blood cell count, mean cell volume, or hematocrit were related to manganese dose in the normal low-iron group. Young animals, 24-100 days of age, on low-iron diets developed microcytic anemia related to manganese dose.

6.6.3. Summary. Reports of hematological effects are conflicting, but increased hemoglobin values and erythrocyte counts have been associated with human (Kesic and Häusler, 1954) and animal (Baxter et al., 1965) exposures to high levels of manganese. Young animals maintained on a low-iron diet and receiving manganese treatment during the prenatal and postnatal periods may develop a microcystic anemia (Carter et al., 1980).

6.7. CARDIOVASCULAR SYSTEM EFFECTS

6.7.1. Human Studies. Saric and Hrustic (1975) measured blood pressure in three groups of workers aged 20-59 to observe the effect of exposure to airborne manganese. The diastolic and systolic blood pressure of 367 exposed workers from a ferromanganese plant were compared to 189 workers in electrode production within the same plant not directly exposed to manganese, and 203 workers in a light metal plant unexposed to manganese. Seventy-five percent of exposed workers had been exposed for more than 4 years. The mean concentration of manganese for work sites with manganese alloy varied from 0.39-20.44 mg/m³. At sites for electrode production, the concentrations varied from 0.002-0.30 mg/m³.

Workers in the manganese alloy plant had the lowest mean systolic blood pressure (130.8) followed by electrode plant workers (133.6) and the light metal plant workers (138.7). The same trend occurred in each of four 10-year age groups and in all workers excluding hypertensives. The lowest mean diastolic pressure was in workers in the light metal plant, followed by the manganese alloy plant workers and then those from the electrode plant.

This was observed also for each age group except the oldest and was also seen when hypertensives were excluded. All of the comparisons were significant at the 0.05 or 0.01 level, but since multiple t-tests were performed, this should be interpreted with caution. It has to be noted that although the mean body weight in the compared groups did not differ, a detailed analysis of the body bulk in relation to the blood pressure values was not performed. As stated by the authors, other risk factors also may have been insufficiently controlled. Saric (1978) suggests that the differences found in the behavior of systolic and diastolic blood pressure in those occupationally exposed to manganese may indicate an action of manganese ions on the myocardium.

6.7.2. **Animal Studies.** In rats, Kimura et al. (1978) found that dietary exposure to 564 ppm manganese produced a significant increase in the level of blood serotonin and a decrease in blood pressure. The researchers attributed the final marked decrease of blood pressure to the elevated concentration of serotonin in the blood, probably released from different tissues.

6.7.3. **Summary.** Manganese exposure has elicited decreases in systolic blood pressure in humans (Saric and Hrustic, 1975) and in animals (Kimura et al., 1978). This latter finding was attributed to the elevated concentration of serotonin in the blood.

6.8. BIOCHEMICAL EFFECTS

6.8.1. **Human Studies.** Rodier (1955) reported diminished excretion of 17-ketosteroids in 81% of the patients with chronic manganese poisoning and an increase in basal metabolism in 57% of the cases with manganism. These conclusions are reported with no supporting data.

postnatal period, no changes in red blood cell count, mean cell volume, or hematocrit were related to manganese dose in the normal low-iron group. Young animals, 24-100 days of age, on low-iron diets developed microcytic anemia related to manganese dose.

6.6.3. Summary. Reports of hematological effects are conflicting, but increased hemoglobin values and erythrocyte counts have been associated with human (Kesic and Häusler, 1954) and animal (Baxter et al., 1965) exposures to high levels of manganese. Young animals maintained on a low-iron diet and receiving manganese treatment during the prenatal and postnatal periods may develop a microcystic anemia (Carter et al., 1980).

6.7. CARDIOVASCULAR SYSTEM EFFECTS

6.7.1. Human Studies. Saric and Hrustic (1975) measured blood pressure in three groups of workers aged 20-59 to observe the effect of exposure to airborne manganese. The diastolic and systolic blood pressure of 367 exposed workers from a ferromanganese plant were compared to 189 workers in electrode production within the same plant not directly exposed to manganese, and 203 workers in a light metal plant unexposed to manganese. Seventy-five percent of exposed workers had been exposed for more than 4 years. The mean concentration of manganese for work sites with manganese alloy varied from 0.39-20.44 mg/m³. At sites for electrode production, the concentrations varied from 0.002-0.30 mg/m³.

Workers in the manganese alloy plant had the lowest mean systolic blood pressure (130.8) followed by electrode plant workers (133.6) and the light metal plant workers (138.7). The same trend occurred in each of four 10-year age groups and in all workers excluding hypertensives. The lowest mean diastolic pressure was in workers in the light metal plant, followed by the manganese alloy plant workers and then those from the electrode plant.

Jonderko et al. (1971) compared a group of manganese-exposed workers who did not exhibit symptoms or signs of intoxication with a control group of 45 workers. The exposed workers had lower levels of magnesium, hemoglobin, and reduced glutathione, while calcium and cholesterol levels were increased. In an evaluation of the effects of manganese exposure on the development of atherosclerosis, several variables were compared between 110 workers in a steel mill and 80 nonexposed controls (Jonderko et al., 1973). Workers were exposed for an average of 9 years to values of manganese that were 1.3-50 times above the maximum allowable concentration. The English abstract published with this study reported statistically significant increases in mean cholesterol, β-lipoproteins and total lipoproteins, as well as increased incidences of hypertension and atherosclerosis in the exposed group. However, there is no stratification or other control for confounding variables such as smoking or obesity. The information available from table headings and the abstract did not describe exposure levels or age distribution and the statistical test was not named in English.

Jonderko et al. (1974) also examined a group of 34 iron-manganese plant workers during employment and 2-4 years after cessation of occupational exposure. When compared with a group of 45 control subjects, Jonderko found slight changes with a tendency to normalization after exposure ceased in a number of biochemical parameters, including lactate dehydrogenase, alanine and asparagine aminotransferase, cholesterol, and glutathione levels. Hemoglobin concentration in the followed workers also increased from 12.6 during employment to 13.9 in the follow-up.

In a clinical and biochemical study conducted in 12 cases of suspected manganese poisoning, Chandra et al. (1974) reported a statistically significant increase in serum calcium and adenosine deaminase levels in cases of

mild and moderate grades of poisoning, and particularly in a case of severe poisoning, compared with values in normal volunteers. They suggest that serum calcium levels be used to detect manganese poisoning in the early stages.

6.8.2. Animal Studies. Intratracheal administration of 400 mg MnO_2/kg bw to rats caused a significant decrease in the levels of serum alkaline phosphatase and inorganic phosphate, and an increase in calcium (Chandra et al., 1973b). Similar observations were reported by Jonderko (1965): Rabbits injected intramuscularly with 3.5 mg Mn/kg bw showed a distinct increase of serum calcium and a decrease of inorganic phosphorus. However, the mechanism of hypercalcaemia and hypophosphataemia in manganese toxicity was not clear because no gross or microscopic abnormalities were observed in parathyroids and bones of exposed rats (Chandra et al., 1973b).

Chandra and Imam (1975) studied the effect of i.v. administered 2.5 mg $MnCl_2$/kg bw on the rabbit adrenal cortex. An increase in the cholesterol content and marked degenerative changes in the zona glomeruloza and zona fasciculata were observed after a period of 2 months. Three months after the beginning of exposure, the damaging effect of manganese on the adrenal cortex was even more marked.

6.8.3. Summary. Effects of manganese exposure on the biochemical parameters include an increase in serum calcium, adenosine deaminase, cholesterol, total lipids and β-lipoproteins in workers occupationally exposed to manganese (Jonderko et al., 1974). A diminished excretion of 17-ketosteroids has been reported in patients with chronic manganese poisoning. Animal experiments demonstrate a decrease in the levels of serum alkaline phosphatase and inorganic phosphate, and an increase in calcium in manganese toxicity (Chandra et al., 1973b).

6.9. DIGESTIVE SYSTEM EFFECTS

6.9.1. Gastrointestinal Tract Effects. The paucity of data and the controversy regarding the doses used in the available studies cause great difficulty in assessing toxic effects of manganese on the GI tract. For example, Chandra and Imam (1973) described significant histochemical and histological alterations in the GI mucosa of guinea pigs exposed orally to ~4.4 mg Mn/kg bw for a period of 30 days. However, an amount of ~4 mg Mn/kg bw has been recommended by the NAS (1973) as a minimum requirement for guinea pigs. Even though no specific effort was directed to determine the minimum manganese daily requirements, Everson et al. (1959) reported a diet to be adequate with the presence of 40 ppm manganese. Further, Shrader and Everson (1968) reported that manganese supplementation (125 ppm for 2 months) completely reversed the reduced glucose utilization caused by congenital manganese deficiency.

6.9.2. Liver Effects. The liver plays a significant role in manganese metabolism, and the biliary route is very important for the removal of manganese from the body. Over 99% of an i.v. dose excreted by the rat appeared in the feces (Klaassen, 1974). However, manganese has produced intrahepatic cholestasis in rats, with large doses causing both functional and morphological alterations (Witzleben et al., 1968; Witzleben, 1972). An i.v. dose of 55-60 mg/kg bw manganese caused necrosis in rat liver and other ultrastructural alterations resembling some of those seen in human cholestasis induced by drugs (Witzleben, 1969). When manganese overload was followed by infusion of bilirubin, the lesions were even more severe (Witzleben, 1971, 1972), depending upon the dose of bilirubin (Boyce and Witzleben, 1973).

Klaassen (1974) reported that no alteration in the bile flow was observed in rats even at the relatively high i.v. dose of 10 mg Mn/kg bw.

However, when bilirubin was administered immediately after manganese injection, there was an almost complete cessation of bile flow, even at small doses of manganese (3 mg Mn/kg) which are not cholestatic when given alone. The researcher suggested the possibility that bilirubin may form a chelate with manganese which precipitates and obstructs the biliary tree.

De Lamirande and Plaa (1978, 1979a,b) showed in a series of experiments on rats that both manganese and bilirubin are essential for the induction of cholestasis. Small noncholestatic doses of each resulted in cholestasis when given together, but the order and time of injection were critical. These observations suggest that the manganese-bilirubin interaction might depend on the presence of short-lived intermediate compounds during the process of manganese biliary excretion.

In an attempt to study the ultrastructural changes in the liver using doses known to be nontoxic, Wassermann and Wassermann (1977) gave rats drinking water with an extra dosage of 200 ppm $MnCl_2$. The ultrastructural changes found were an increased amount of rough endoplasmic reticulum, a proliferated smooth endoplasmic reticulum, prominent Golgi apparatuses and the occurrence of multiple rough endoplasmic cisternae, which may be interpreted as an adaptation process to increased exposure to $MnCl_2$.

Various biochemical or histological changes in the liver were reported in a number of studies, mainly as side effects in the experiments where neurological, respiratory, or reproductive effects of manganese were investigated. Chandra and Tandon (1973) and Shukla et al. (1978) reported some biochemical and histopathological alterations in the livers of rats given orally 2.8 or 4.4 mg Mn/kg bw. However, as was stressed earlier (see Section 4.3.2.3.), the administered doses were too low to be considered toxic to rats. Thus, the rats on manganese-supplemented diets (564 ppm manganese)

did not manifest abnormalities in the liver, and the liver monoamine oxidase activity remained the same as in the control group of animals (Kimura et al., 1978).

Parenteral administration of manganese sulfate in a dose of 6 mg Mn/kg bw did not significantly affect the enzyme activity in the liver of exposed rats, in spite of a significant accumulation of this metal in the liver (Singh et al., 1974, 1975). Only the activity of succinic dehydrogenase and lactate dehydrogenase decreased to a considerable extent. Some pathomorphological alterations were observed in the liver of the treated animals, where some of the sections showed mild congestion of central veins and adjacent sinusoids. Minute areas of focal necrosis were noticed throughout the section.

Microscopic examination of the liver in monkeys exposed parenterally to relatively high doses of manganese showed only mild changes. In monkeys receiving 345 mg Mn/kg bw, Pentschew et al. (1963) found only hemosiderosis of the Kupffer cells. Neff et al. (1969) described only variable, often mild, vacuolar changes in the liver cells of the monkeys injected s.c. with 500 mg Mn/kg bw. Finally, Suzuki et al. (1975) reported that an irregular arrangement of hepatic cords and lymphocytic infiltration of Glisson's capsules were seen in two monkeys receiving the highest doses, totaling 5680 mg Mn/kg bw over a period of 9 consecutive weeks.

6.9.3. Summary. The lack of data and the controversy over the doses used in the available studies cause difficulty in assessing toxic effects of manganese on the intestine. On the other hand, more data are available about the hepatotoxic effects of manganese. The liver plays a significant role in manganese metabolism, and the biliary route is very important for the removal of manganese from the body. Over 99% of the i.v. dose was excreted

by the rat in the feces. Manganese has been described as an agent that produces intrahepatic cholestasis, large doses causing both functional and morphological alterations. An i.v. dose of manganese at a concentration of 55-60 mg/kg bw of the rat caused necrosis in the liver and other ultrastructural alterations resembling some of those seen in human cholestasis induced by drugs (Witzleben, 1969). Microscopic examination of the liver in monkeys exposed parenterally to relatively high doses of manganese showed only mild changes, for example, hemosiderosis of the Kupffer cells.

7. CARCINOGENICITY

7.1. ANIMAL STUDIES

Manganese sulfate in sodium chloride has been tested for carcinogenic activity in the Strain A mouse lung tumor system (Stoner et al., 1976). In this study, A/Strong mice of both sexes, 6-8 weeks old, were injected intraperitoneally 3 times/week for a total of 22 injections. Three dose levels were employed that represented the maximum tolerated dose, a 1:2 dilution and a 1:5 dilution of the maximum tolerated dose. Twenty mice were used at each dose level (10/sex) including vehicle (saline) and positive (urethan) controls. Mice were sacrificed 30 weeks after the first injection, and the frequency of lung tumors in each test group was statistically compared with that in the vehicle-treated controls using the student t test.

The interpretation of the lung tumor data in the Strain A mouse is somewhat unusual in that certain specific criteria should be met before a compound is considered positive (Shimkin and Stoner, 1975):

1. A significant increase in the mean number of lung tumors in test animals, preferably $\geq$1/mouse, should be obtained;
2. A dose-response relationship should be evident.
3. The mean number of lung tumors in control mice should be consistent with the anticipated incidence of spontaneous tumors for untreated strain A mice.

The results obtained by Stoner et al. (1976) are summarized in Table 7-1. These data indicate that the above criteria were not conclusively met for the establishment of a positive response. A slight but statistically significant increase in the number of pulmonary adenomas per mouse was associated with administration of the high dose. The response was somewhat elevated at the other doses, but was not statistically significant. Overall, it can be concluded that the results of this experiment are suggestive of carcinogenic activity.

TABLE 7-1

Pulmonary Tumors in Strain A Mice Treated with Manganese Sulfate[a]

Group	Total Dose mg $MnSO_4$/kg	Total Dose mg Mn/kg	Mortality	Mice with Lung Tumors (%)		Average Number Tumors/Mouse[b]	p[c]
Untreated control	0	0	1/20	6/19	(31)	0.28±0.07	NA
Solvent control (0.85% NaCl)	0	0	1/20	7/19	(37)	0.42±0.10	NA
Treated	132	42.9	1/20	7/19	(37)	0.47±0.11	NS
Treated	330	107.2	0/20	7/20	(35)	0.65±0.15	NS
Treated	660	214.4	2/20	12/18	(67)	1.20±0.49	0.05[d]
20 mg urethan[e]	0	0	2/20	18/18	(100)	21.6±2.31	NR

[a]Source: Stoner et al., 1976

[b]X±S.E.

[c]Student t test

[d]Fisher Exact Test p = 0.068

[e]Single intraperitoneal injection

NA = Not applicable; NS = Not significant; NR = Not reported

DiPaolo (1964) injected CBA/J mice subcutaneously or intraperitoneally with 0.1 mℓ of a 1% $MnCl_2$ aqueous solution twice weekly for 6 months. Control mice were injected with water. Mice were sacrificed as they became moribund or at 18 months of age. Sixty-seven percent (24/36) and 41% (16/39) of the mice treated subcutaneously and intraperitoneally, respectively, had lymphosarcomas; the incidence in controls was 24% (16/66). Tumors appeared earlier in the treated groups than in the control group, but statistically significant differences in the number of other tumors (e.g., mammary adenocarcinomas, leukemias, injection site tumors) did not occur. The results of this study were published in abstract form, and additional details regarding experimental design or results were not given. Therefore, a thorough evaluation of the results is not possible.

Furst (1978) evaluated the carcinogenicity of manganese powder and MnO_2 in F344 rats and Swiss mice, and manganese (II) acetylacetonate (MAA) in F344 rats. The test materials were suspended in trioctanoin, and administered intramuscularly (i.m.) or by gavage as follows. Groups of 25 rats of each sex were administered 10 mg manganese (i.m.) per month for 9 months, 10 mg MnO_2 (i.m.) per month for 9 months, 50 mg MAA (i.m.) per month for 6 months, or 10 mg manganese (gavage) twice per month for 12 months. Groups of 25 female mice were administered single 10 mg doses of manganese powder, 6 doses of 3 mg MnO_2, or 6 doses of 5 mg MnO_2 via i.m. injection, but the frequencies of injection in these experiments were not stated. Complete necropsies were performed on all animals and obvious growths, suspicious tissues, lungs and livers were examined histologically. The duration of the experiments were not specifically stated, but were implied to be 2 years in the rat experiments. As summarized in Table 7-2, no difference in tumor incidence was noted between treated and control animals with respect to

TABLE 7-2

Carcinogenicity of Manganese Powder, Manganese Dioxide and Manganese Acetylacetonate in F344 Rats and Swiss Albino Mice[a]

Compound[b]	Species	Route	Treatment Schedule[c]	Total Dose	Tumor Type	Incidence (males)	Incidence (females)
Triglyceride control	rat	i.m.	0.2 ml/month x 12 months	2.4 ml	lymphomas/leukemia fibrosarcomas[d]	1/25 1/25	3/25 1/25
Manganese powder	rat	i.m.	10 mg/month x 9 months	90 mg	lymphomas/leukemia fibrosarcomas	3/25 3/25	5/25 0/25
Manganese acetylacetonate	rat	i.m.	50 mg/month x 6 months	300 mg	lymphomas/leukemia fibrosarcomas other sarcomas	2/25 10/25[e] 3/25[g]	2/25 6/25[f]
Triglyceride control	rat	i.m.	0.2 ml/month x 12 months	2.4 ml	lymphomas/leukemia fibrosarcomas	0/25 0/25	4/25 0/25
Manganese dioxide	rat	i.m.	10 mg/month x 9 months	90 mg	lymphomas/leukemia fibrosarcomas	0/25 0/25	3/25 0/25
Triglyceride control	rat	oral	0.5 ml, twice monthly x 12 months	12.5 ml	lymphomas/leukemia fibrosarcomas	3/25 0/25	3/25 0/25
Manganese powder	rat	oral	10 mg, twice monthly x 12 months	240 mg	lymphomas/leukemia fibrosarcomas	0/25 0/25	0/25 0/25
Triglyceride control	mouse	i.m.	0.2 ml/injection x 3 injections	0.6 ml	leukemia lymphomas	NT NT	2/25 1/25
Manganese powder	mouse	i.m.	10 mg (single injection)	10 mg	leukemia lymphomas	NT NT	6/25 1/25

Table 7-2 (cont.)

Compound[b]	Species	Route	Treatment Schedule[c]	Total Dose	Tumor Type	Incidence (males)	Incidence (females)
Triglyceride control	mouse	i.m.	0.2 mℓ/injection x 12 injections[h]	24 mℓ	leukemia lymphomas	NT NT	2/25 0/25
Manganese dioxide	mouse	i.m.	3 mg/injection x 6 injections[h]	15 mg	leukemia lymphomas	NT NT	4/25 1/25
Manganese dioxide	mouse	i.m.	5 mg/injection x 6 injections[h]	30 mg	leukemia lymphomas	NT NT	1/25 2/25

[a]Source: Furst, 1978

[b]Compounds suspended in 0.2 mℓ (i.m.) or 0.05 mℓ (gavage) trioctanoin

[c]Duration of experiments was not stated, but was implied to be 2 years in the rat studies. The average weights of the treated and control mice ranged from 22-25 g at the start of the experiments to 33-39 g at the end of the experiments.

[d]Injection site fibrosarcoma

[e]Fisher Exact Test p = 0.002

[f]Fisher Exact Test p = 0.049

[g]Incidence includes 2 rhabdomyosarcomas and 1 myxosarcoma

[h]Intervals between injections not stated

NT = Not tested

manganese powder and MnO_2. In contrast, a statistically significant number of fibrosarcomas (10 tumors in 25 male rats, $p = 0.002$; 6 tumors in 25 female rats, $p = 0.049$) developed at the injection site in the rats given MAA as compared to vehicle controls; the mean latency period was 17 months. However, the results of the assay with the organomanganese compound (MAA) cannot necessarily be extrapolated to pure manganese or other inorganic manganese compounds. Furst (1978) commented that MAA suspended well in the vehicle, and that the carcinogenic effect may therefore be inconsistent with foreign-body carcinogenesis. Further, it is doubtful whether these results have any relevance to exposure to inorganic manganese through inhalation.

In June 1980, the Executive Committee of the National Toxicology Program included manganese sulfate in the list of priority chemicals for testing the toxicologic and carcinogenic effects. Prechronic testing of manganese sulfate in Fisher 344 rats and $B6C3F_1$ mice administered via their feed began during March 1982 [National Cancer Institute (NCI), 1982].

Intraperitoneal injection of another organomanganese compound, methylcyclopentadienyl manganese (MMT) (80 mg/kg), produced cell proliferation in the lungs of female A/J mice (Witschi et al., 1981). When mice (30/group) were treated with single injections of urethan (500 mg/kg) followed 1 week later by 6 weekly injections of 80 mg/kg MMT, lung tumor formation was not enhanced when compared with urethan treated controls. Weekly injections of MMT alone did not increase the incidence of spontaneously occurring lung tumors.

Sunderman et al. (1974, 1976) also reported that i.m. administration of manganese did not induce injection site tumors in Fischer rats. Single i.m. injections of 0.5 ml of penicillin suspensions containing manganese dust

were adminstered at the dosages specified in Table 7-3, and incidences of local sarcomas were tabulated after 2 years. The results of other similarly-designed experiments in these studies indicated that addition of equimolar amounts of manganese dust to nickel subsulfide (Ni_3S_2) dust significantly depressed Ni_3S_2-induced tumorigenesis. Subsequent work by the same group of investigators (Sunderman et al., 1980) showed that, under the same experimental conditions, manganese dust also inhibited local sarcoma induction by benzo(a)pyrene.

7.2. HUMAN STUDIES

There are numerous epidemiological studies designed to evaluate the chronic effects of manganese, such as CNS abnormalities or pneumonia, but none have attempted to relate manganese exposure to cancer mortality or incidence. To assess the relationship between manganese in soil and cancer, Marjanen (1969) correlated the amount of soluble manganese in cultivated mineral soil in 199 parishes with the 5-year cancer incidence rates from 1961-1965. He determined that cancer incidence decreased with increasing content of manganese; there was a statistically significant correlation coefficient of -0.66. The data excluded cities and were not age-adjusted. Further work is needed to assess the effect of confounding factors such as age differences among parishes, social class and dietary habits, type of cancer contributing to the association, and extent of consumption of local foods.

Blood plasma levels of manganese were reported to be elevated in patients with stage IV bronchogenic carcinoma (Timaskina et al., 1981). An earlier study (Morgan, 1972) of autopsy samples of hepatic tissue from patients who had died of bronchogenic carcinoma, with and without chronic

TABLE 7-3

Induction of Sarcomas in Rats by the Intramuscular Injection of Manganese Dust

Dosage of Mn Dust[a]	Number of 2-yr Survivors	No. of Rats with Injection Site Sarcomas/Total Number of Rats	Reference
0 mg/rat	16/24	0/24	Sunderman et al., 1974
2.1 mg/rat[b]	17/24	0/24	
0 mg/rat	22/60	0/60	Sunderman et al., 1976
0.5 mg/rat[c]	6/15	0/15	
1.0 mg/rat[c]	2/15	0/15	
2.0 mg/rat[c]	8/15	0/15	
4.0 mg/rat[c]	10/15	0/15	
4.4 mg/rat[d]	NR	0/20	Sunderman et al., 1980
4.4 mg/rat[d]	NR	0/20	

[a]Fischer rats were given a single i.m. injection of 0.5 mℓ of penicillin suspension containing the manganese dust.

[b]Mean particle diameter, 1.4 μm. Manganese dust was composed of 62% elemental Mn, 36% manganese oxides (as MnO_2), <0.1% Ni, Cu, Cr, and Co, 2% Al.

[c]Mean particle diameter 1.6 μm. Manganese dust was composed of 94% elemental Mn; 6% O_2; <0.02% Al, Co, Cu, and Ni; 0.01% Cr.

[d]The results of two 2-year experiments were reported in an abstract, but control data were not reported.

NR = Not reported

bronchitis and emphysema, reported slightly elevated hepatic manganese concentrations in patients with emphysema and carcinoma ($p = 0.05$), but not in patients with emphysema and bronchitis alone or lung carcinoma alone.

Malignant breast tissue concentrates contained significantly higher amounts of copper, magnesium, zinc, and manganese than did noncancerous breast tissue (Mulay et al., 1971). However, a subsequent study measured trace metals in cancerous and noncancerous breast tissue and found only magnesium and zinc levels to be elevated (Santoliquido et al., 1976). Manganese was found to be elevated in osteogenic sarcoma tissue when compared to normal specimens (Leach, 1971; Jones et al., 1972).

The remainder of the studies pertaining to manganese levels in cancerous tissue relate to manganese-superoxide dismutase. Superoxide is an anionic free radical and an active reducing agent. Superoxide dismutases (SOD) convert superoxide to H_2O_2, which in turn is converted to water by catalase and peroxidase (Fee, 1980). Two types of SOD are found in eukaryotic cells: Zn/Cu SOD in the cytoplasm and Mn SOD within mitochondria (Sun et al., 1980; Oberly and Buettner, 1979). Mn SOD is generally reduced or absent in tumor mitochondria, including mouse neuroblastoma cells (Oberly et al., 1978), rat Morris hepatoma (Bize and Oberly, 1979; Bize et al., 1980), rat hepatoma HC-252 (Sun et al., 1980), and human lymphoma lymphocytes (Issels and Lengfelder, 1981). Other human tumors (Westman and Marklund, 1981) and chemically-induced rat colon adenocarcinoma (Loven et al., 1980), however, were not found to have decreased levels of Mn SOD.

Diminished Mn SOD is correlated with increased production of superoxide radicals; manganese has been suggested as a dietary supplement in cancer treatment, particularly for protection against the extra superoxide produced by activated macrophages involved in antitumor immunity (McCarty, 1981).

Reduced levels of Mn SOD have been hypothesized to prevent differentiation of cancer cells due to increased superoxide, and addition of SOD to transformed cells seems to overcome some of the blockage of cell differentiation (Oberly et al., 1980).

7.3. SUMMARY

Repeated subcutaneous or intraperitoneal (i.p.) injections of manganese dichloride induced increased incidences of lymphosarcomas in DBA/1 mice, and manganous sulfate (i.p.) elicited suggestive results in a strain A mouse lung tumor bioassay. Intramuscular injections of MnO_2 or manganese powder did not induce a statistically significant increased incidence of lymphosarcomas, leukemias or local sarcomas in either sex of F344 rats or female Swiss mice, and oral administration of manganese powder for 12 months did not produce lymphomas, leukemias or fibromas in either sex of F344 rat. Intramuscular injection of manganese acetylacetonate resulted in a statistically significant increased incidence of injection site fibrosarcomas in both sexes of F344 rats. Although the results of the studies with divalent manganese were probably suggestive of carcinogenic activity, it should be emphasized that non-natural routes of administration were employed.

There is some evidence of carcinogenic activity of manganese in laboratory animals in the literature, although problems exist with regard to the value of these studies (i.e., local injection site sarcomas in F344 rats, a marginal response in strain A mice, and inadequate data in the experiment with DBA/1 mice). There is no epidemiologic information relating manganese exposure to cancer occurrence in humans.

In conclusion, the available evidence for manganese carcinogenicity in humans would be rated Group 3 overall using the International Agency for Research on Cancer (IARC) criteria, because of inadequate data in animals and lack of any available data in humans. Clearly, more information is needed before a more definitive conclusion can be made about the carcinogenicity of manganese and its compounds.

8. MUTAGENICITY AND TERATOGENICITY

8.1. MUTAGENICITY

A preliminary review of the currently available mutagenicity data has been performed. The data are both insufficient and inadequate at this time to reach a conclusion about the mutagenic potential of manganese.

8.2. TERATOGENICITY

In animals, manganese deficiency during pregnancy causes a variety of developmental defects related to decreased formation of chondroitin sulfate and delayed otolith calcification. Resultant defects included reduced coordination, bone and growth deficiencies, reproductive difficulties, and CNS changes (Oberleas and Caldwell, 1981; Hurley, 1981). The effect of manganese excess has been studied by only a few investigators.

In rodents, excess manganese during pregnancy affects behavioral parameters, as described in two recent abstracts. Hoshishima et al. (1978) reported that geotaxis performance, but not intelligence testing, was impaired in mice treated in utero with manganese. In another study, Massaro et al. (1980) exposed female mice from days 0 through 18 of pregnancy with MnO_2 dust (48.9 ± 7.5 mg/m^3, continuous exposure). Litters from exposed and nonexposed mothers were reduced to three pups of each sex, and the pups were fostered equally among exposed and nonexposed mothers. Pup weight and activity were not different whether or not they had been exposed in utero, but as adults exposed pups were deficient in open-field, exploratory, and rotarod (balance and coordination) performance. Normal offspring fostered to exposed mothers also showed decreased rotarod performance, indicating that post-partum exposure can also have an adverse effect on behavioral development. This is supported by the effect of manganese on learning in the adult rat (Murthy et al., 1981), and by a study of the distribution of

^{54}Mn in fetal, young, and adult rats. Early neonates and 19-day fetuses were more susceptible to manganese than the older groups; manganese localized to the liver and brain in the younger groups and they accumulated more manganese per weight than the older groups (Kaur et al., 1980). No fetal abnormalities were seen when 18-day embryos were exposed to 16 μmol/200 g maternal weight, but this is a late stage for detecting developmental defects.

8.3. SUMMARY

Although data reported in abstracts suggest that excess manganese during pregnancy affects behavioral parameters, there is insufficient evidence to define manganese as being teratogenic.

9. EFFECTS OF CONCERN AND HEALTH HAZARD EVALUATION

9.1. EXISTING GUIDELINES, RECOMMENDATIONS AND STANDARDS

9.1.1. Air. In the United States, the American Conference of Governmental and Industrial Hygienists (ACGIH, 1980) has recommended 5 mg/m^3 as both the time-weighted average threshold limit value (TWA-TLV) and the short-term exposure limit for manganese. This value is based on observations of poisoning in humans at concentrations near or above the recommended TLV. The National Institute for Occupational Safety and Health (NIOSH) has not recommended an occupational criterion for exposure to airborne manganese, and the Occupational Safety and Health Administration (OSHA) has not promulgated a standard for manganese exposure. Occupational standards in some other countries, as summarized by the International Labour Office (ILO, 1980), are listed below:

Country	mg Mn/m^3	Comment
Belgium	5	ceiling value
Czechoslovakia	2 6	ceiling value
Japan	5	
Poland	0.3	
Roumania	1 3	ceiling value
Switzerland	5	ceiling value
USSR	0.3	

The World Health Organization (WHO, 1981) recommends a criterion of 0.3 mg/m^3 for respirable manganese in occupational exposures.

9.1.2. Water. No toxicity-based criteria or standards for manganese in freshwater have been proposed. The WHO (1970), the U.S. PHS (1962), and the U.S. EPA (1976) recommended a concentration of 0.05 mg/ℓ in water to